



**University of Wolverhampton**  
**Department of Biomedical Sciences**

**METABOLISM AND BODY COMPOSITION IN CHRONIC  
INFLAMMATORY ARTHRITIS: PREVENTION  
AND INTERVENTION THROUGH PHARMACEUTICAL AND  
PHYSICAL MEANS**

**A thesis submitted to the University of Wolverhampton for the  
Doctor of Philosophy**

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**March 2007**

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## LIST OF ABBREVIATIONS

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RA	Rheumatoid Arthritis
TNF $\alpha$	Tumour Necrosis Factor alpha
IL-1	Interleukin 1
IL-6	Interleukin 6
REE	Resting Energy Expenditure
FFM	Fat-Free Mass
CRP	C Reactive Protein
ESR	Erythrocyte Sedimentation Rate
DAS28	Disease Activity Score 28
HAQ	Health Assessment Questionnaire
TEF	Thermic Effect of Feeding
TDEE	Total Daily Energy Expenditure
MET	Standard Metabolic Equivalent
ACT	Physical Activity
FFMm	Metabolically Active Fat-Free Mass
FFMnm	Metabolically Inactive Fat-Free Mass
DNA	Deoxyribonucleic acid
HIV	Human Immunodeficiency Virus
ATP	Adenosine tri-phosphate
ARC	Arthritis Research Campaign
BSR	British Society of Rheumatology
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
DMARDs	Disease Modifying Anti-Rheumatic Drugs
BCM	Body Cell Mass
BMI	Body Mass Index
NHS	National Health Service
IPAQ	International Physical Activity Questionnaire
SPSS	Statistical Package for the Social Sciences
LIM <sub>AG</sub>	95% Limits of Agreement
CV%	Percent Coefficients of Variation
Std	Standard deviation
kcal	Kilocalories
ANCOVA	Analysis of Co-Variance
ANOVA	Analysis of Variance

## ACKNOWLEDGMENTS

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This Doctor of Philosophy degree is dedicated to my family: my father Spiridon Metsios, my mother Anastasia Metsiou and my sister Elina Metsiou, who supported me all these years in order to accomplish my studies.

I would also like to thank very much my friend and close colleague Antonis Stavropoulos-Kalinoglou who helped me considerably with my PhD. Moreover, I want to thank my Supervisors Professor Yiannis Koutedakis and Professor George D. Kitas for their faith and great support on this research project, and Professor Alan Nevill who was my statistical advisor and help me very much with the data analyses in order to publish the work that arise from my PhD.

I would also like to express my gratitude for all the personnel in Russell's Hall Hospital, particularly Debbie Mitton, who helped us with the recruitment of the patients, as well as all the other nurses and the personnel who provided significant help in the clinical assessment of the patients and management of the whole project.

Finally, I would like to say a big thanks to my endeared companion Theodora Tsetsou, whose everyday patience and meaningful support reinforced my effort to succeed.



## **RESPONSIBILITIES**

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My responsibilities in the three projects that constituted this Thesis were:

- Structure of the exact methodology
- Writing and gaining the ethical approval form the Dudley Group of Hospitals Ethics Committee, NHS Trust
- Recruitment of patients from the clinics in Corbett Hospital, Stourbridge and Russell's Hall Hospital, Dudley
- Assessment of patients
- Data input. For the analysis and interpretation of the data my supervisors Prof Koutedakis, Prof Kitas and Prof Nevill helped me significantly
- Writing and publishing the results in scientific congresses and peer-reviewed journals.

## ABSTRACT

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**Background:** Rheumatoid arthritis (RA) is characterised by excessive production of tumour necrosis factor alpha (TNF $\alpha$ ). This leads to rheumatoid cachexia, a condition characterised by increased resting energy expenditure (REE) and loss of fat-free mass (FFM) leading to functional disability, decreased strength and balance. The aims of this research work was to: a) to develop a new REE equation in order to continuously monitor abnormal changes in REE in the RA population, b) to investigate if smoking further enhances hypermetabolism and c) to examine if the new anti-TNF $\alpha$  medication reverses this metabolic abnormality. **Methods:** 68 patients with RA were assessed for demographic and anthropometrical characteristics, REE (indirect calorimetry), body composition (bioelectrical impedance), and disease activity [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity score 28 (DAS28) and health assessment questionnaire (HAQ)]. 20 of the total 68 patients, about to start anti-TNF $\alpha$  therapy, underwent the exact same aforementioned procedures but on three separate occasions (Baseline: two weeks prior to anti-TNF $\alpha$  treatment, Time-1 and Time-2: two weeks and three months, respectively, after the drug had been introduced. **Results:** *Study 1:* Based on FFM and CRP, a new equation was developed which had a prediction power of  $R^2=0.76$ . The new equation revealed an almost identical mean with measured REE ( $1645.2\pm315.2$  and  $1645.5\pm363.1$  kcal/day,  $p>0.05$ ), and a correlation coefficient of  $r=0.87$  ( $p=0.001$ ). *Study 2:* Smokers with RA demonstrated significantly higher REE ( $1513.9\pm263.3$  vs.  $1718.1\pm209.2$  kcal/day;  $p=0.000$ ) and worse HAQ ( $1.0\pm0.8$  vs.  $1.7\pm0.8$ ;  $p=0.01$ ) compared to age and FFM matched RA non-smokers. The REE difference was significantly predicted by the interaction smoking/gender ( $p=0.04$ ). *Study*

3: Significant increases were observed in REE ( $p=0.002$ ), physical activity ( $p=0.001$ ) and protein intake ( $p=0.001$ ) between the three times of assessment. Moreover, disease activity significantly reduced [ESR ( $p=0.002$ ), DAS28 ( $p=0.000$ ), HAQ ( $p=0.000$ ) and TNF $\alpha$  ( $p=0.024$ )] while FFM and total body fat did not change (both at  $p>0.05$ ). Physical activity and protein intake were found to be significant within-subject factors for the observed REE elevation after 12-weeks on anti-TNF $\alpha$  treatment ( $p=0.001$  and  $p=0.024$ , respectively). **Conclusions:** Findings from the first study revealed that the newly developed REE equation provides an accurate prediction of REE in RA patients. Moreover, the results from the second study showed that cigarette smoking further increases REE in patients with RA and has a negative impact on patients' self-reported functional status. Finally, our data from the third study suggest that REE remains elevated not because of the maintenance of the RA-related hypermetabolism but due to the concomitant significant increases in physical activity and protein intake.

## 1. CHAPTER 1: INTRODUCTION

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Metabolism is the biochemical process of modifying chemical compounds in living cells. It is through this process that organisms transform nutrients into biochemical tools and structures needed for the maintenance of the living state. Metabolism has two distinct divisions: anabolism and catabolism. In the former, the cell uses energy to construct complex molecules and perform other life functions; in the latter, the cell breaks down complex molecules to yield energy. Because it is counterproductive to have anabolic and catabolic processes occurring simultaneously, there are many signals/stimuli that switch on anabolic processes while switching off the catabolic equivalents and *vice versa*. One such stimulus is chronic disease.

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints; it may also cause inflammation of the tissues around the joints, as well as other organs in the body. The prevalence of RA is 0.5 – 1% in Europe and North America (Symmons et al. 2002), which makes it the commonest chronic inflammatory joint disease. Substantial evidence in the literature has revealed that RA is accompanied by alterations in the normal metabolic processes due to the systemic involvement of this disease (Metsios et al. 2006; Rall and Roubenoff 1996; Rall and Roubenoff 2004; Roubenoff et al. 1994; Roubenoff et al. 1992). In particular, RA-related systemic biochemical processes, such as increased pro-inflammatory cytokine production, prohibit anabolism and enhance catabolism. Altered metabolism, however, has a major impact on disease outcomes, significantly affects human health and leads to increased mortality (Rall and Roubenoff 2004; Roubenoff et al. 1994). This is the reason that the measurement of metabolism is important in terms of prevention and prognosis of adverse disease outcomes.

In the following pages, the author discusses in detail the most important mediator of human metabolism the resting energy expenditure (REE). The author provides information regarding the components of the human body that mediate REE as well as the effects of exercise on REE and examines the differences between individuals with different ages, genders and weight. Moreover, the author comments on the effects of various other stimuli on REE, particularly focusing on the effects of chronic diseases. Given that this PhD Thesis arises from research work based on REE and RA, the author discusses the aetiology and pathogenesis of the disease and focus on its effects on resting metabolism; the author explains in detail the mechanisms that drive the evident RA-related hypermetabolism, as well as the potential interventions that may reverse it. The important questions that arise from the following review of the literature led our research team to conduct research into the interesting area of human applied physiology. Our hypotheses, methods, and results along with the clinical significance of our findings are discussed in detail in the relevant Chapters and the General Discussion.

## 2. CHAPTER 2: REVIEW OF THE LITERATURE

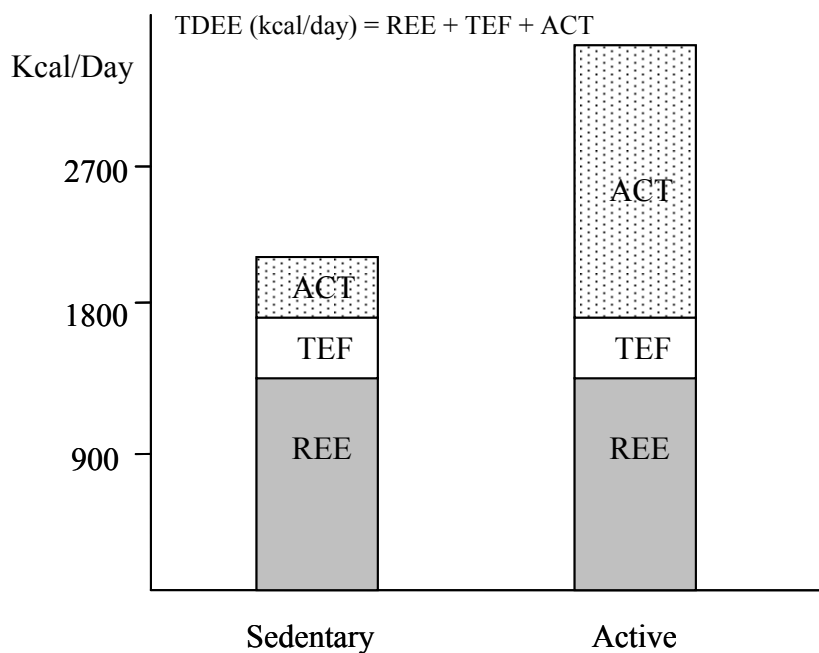
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Parts of the Review of the Literature have been published in the journal *Hospital Chronicles*, 1(1):20-26.

### 2.1 Resting Energy Expenditure

Total daily energy expenditure (Figure 1) is composed of three major components: REE (accounting for ~70%), thermic effect of feeding (TEF) and physical activity [(each accounting for ~ 15%), (Poehlman 1989)]:

**Figure 1.** Components of total daily energy expenditure.



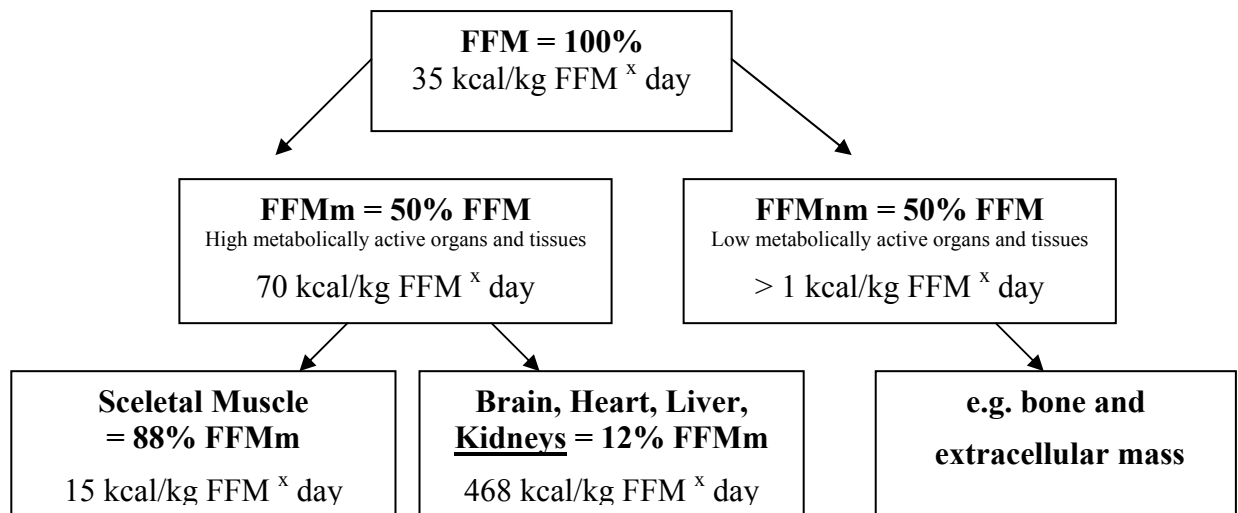
where TDEE = total daily energy expenditure; REE = resting energy expenditure; TEF = thermic effect of feeding; ACT = physical activity

REE, which is also referred to as resting metabolic rate or basal metabolic rate is the minimum level of energy that the body needs to sustain its vital functions (Wilmore and Costill 2004). The laboratory assessment of REE requires the participant to be in a state of physical and mental rest, lying on a comfortable bed in a thermoregulated room, after refraining from strenuous physical activity for at least 72 hours. Moreover, the participant has to be fasted for at least 12 hours.

Specific organs and tissues have a significant impact on REE while fat-free mass (FFM) is the major determinant of REE. FFM is divided in high (e.g., liver, brain) and low (e.g., bones) metabolically active tissue, each having significant differences in the metabolic cost (Figure 2). In contrast, fat mass is a relatively inactive tissue and has a relatively small metabolic expenditure [(~ 4%) (Muller et al. 2004; Wang et al. 2000)].

To determine the contribution of various factors affecting REE, a study (Rodriguez et al. 2002) examined REE differences between obese and non-obese children and adolescents using multiple regression analysis (with REE as dependent variable). Results revealed that REE was significantly higher in obese than in non-obese individuals but REE/FFM ratio was not significantly different between the two groups. In the non-obese group, FFM explained 73.1% of the variability in REE with gender, age and surface area adding 3.8%, 2.6%, and 2.6% to it, respectively. In the obese group, FFM was also the most powerful predictor of REE with 72.3%, followed by waist circumference and age with 2.5% and 2.1%, respectively. These results show that FFM significantly affects REE, thus, establishing FFM as the major determinant of human resting metabolism.

**Figure 2.** Fat-free mass and energy expenditure.



Note: FFMm: metabolically active fat-free mass; FFMnm: low metabolic rate fat-free mass.

## 2.2 Changes in Resting Energy Expenditure

Literature findings suggest that REE varies among individuals, a phenomenon which is explained by factors such as age, sex, body size and composition (as a result of changes in FFM), physical activity and diet as well as a result of chronic disease.

### 2.2.1 Ageing and Resting Energy Expenditure

It has been well established that FFM and REE decrease and fat mass increases with age (Ruggiero and Ferrucci 2006). A recent meta-analysis concludes that both total daily energy expenditure and REE progressively decrease as a result of ageing (Roberts and Dallal 2005) mostly due to concomitant decreases in FFM (Barlett et al. 1991; Bosy-Westphal et al. 2003; Flynn et al. 1989; Keys et al. 1973; Kyle et al. 2001; Poehlman et al. 1993). It is worth noting that REE is significantly lower in older compared to younger



adults even after adjustment for differences in fat mass and FFM (Fukagawa et al. 1990; Hunter et al. 2001; Klausen et al. 1997; Morgan and York 1983; Visser et al. 1995). This indicates that decreases in REE may also occur even if FFM remains unchanged suggesting that resting metabolism may decrease through other mechanisms yet to be clearly defined.

Apart from the reduction in FFM, REE in older individuals has been linked with free-radicals. Free-radicals are highly reactive agents that cause physiological damage (Speakman 2005). Oxygen free-radicals (such as superoxide, hydrogen peroxide, and hydroxyl radical) are formed as a by-product of oxidative phosphorylation, the largest source of free-radicals (Golden and Melov 2001). Despite the development of defensive mechanisms by the human body to protect the damage from free-radicals and ameliorate their effect, some damage always occurs; a consequence is the accumulation of oxidation that leads to physiological attrition and failure (Beckman and Ames 1998; Sohal and Weindruch 1996). Animal studies reveal that the production of free-radicals from mitochondria correlates positively with REE (Ku and Sohal 1993). It could be suggested, therefore, that abnormal increases in oxygen consumption (i.e., abnormally elevated metabolism due to disease) will result in an increase in the formation of radical oxygen species.

#### *Exercise and Free Radicals*

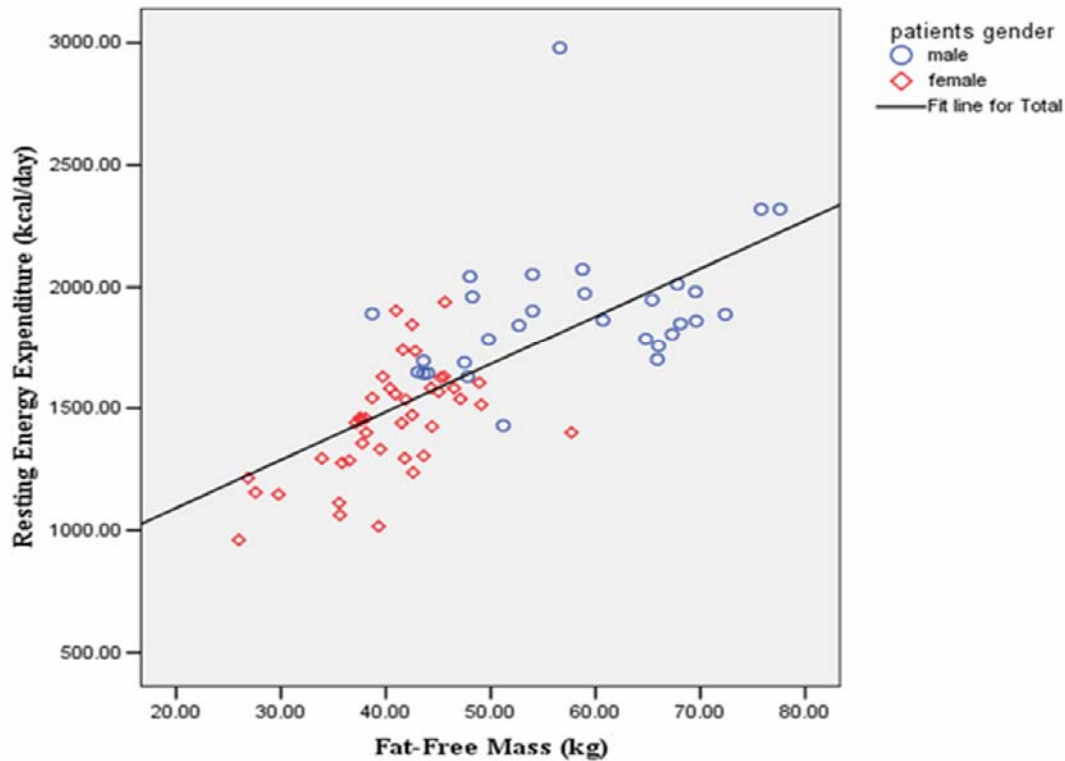
It has been well established that free radicals increase in response to exercise, causing damage to lipids, proteins and DNA. For example, a single bout of moderate exercise increases oxidative stress (Davison et al. 2006). This exercised-induced increase in free radicals is due to the increased electron leak from the mitochondria as well as the alterations in blood flow and oxygen supply that occur in response to exercise (Urso and

Clarkson 2003). Despite the fact that regular physical activity improves the body's antioxidant defense mechanisms (i.e. increases in glutathione peroxidase and superoxide dismutase), antioxidant supplementation after exhaustive exercise does not seem to ameliorate the damage caused by free radicals (Davison et al. 2005). In general, regular exercise upregulates antioxidant enzymes and also reduces the risk for cardiovascular disease and cancer (Friedenreich and Orenstein 2002; Ornish et al. 1998), diseases which have been linked to increased production of free radicals (Sigurdardottir et al. 2002; Weitzman et al. 1994).

### 2.2.2 Gender and Resting Energy Expenditure

Men exhibit greater REE values than women (Poehlman 1989), suggesting that gender is a factor influencing REE. The differences, however, are only observed in adults, since gender has no effect on energy expenditure in prepubertal children; FFM before adulthood is similar in both genders (Grund et al. 2000). During the first years of adolescence and until the 18<sup>th</sup> year of age, REE constantly increases whereas the difference between genders at the 18<sup>th</sup> year of age can be as much as 122 - 131 kcal/day (Tershakovec et al. 2002). From that stage, women tend to have lower REE than men (Figure 3), explained mainly by differences in FFM between the two sexes. This is why after standardising for FFM, REE difference between genders appears to be non-significant (Nielsen et al. 2003). Women experience a further reduction in REE shortly after menopause (Matthews et al. 1989; Poehlman 2005). This decrease is approximately 100 kcal/day in the first year, during which FFM of women has been reported to decrease accordingly (Poehlman et al. 1995b).

**Figure 3.** Comparison of resting energy expenditure in males and females.



### 2.2.3 Body Mass and Resting Energy expenditure

Differences in body mass [and body surface area (term used mainly in medicine for the prescription of medicine)] account for differences in REE due to strong associations between body size and FFM (McArdle et al. 2001). It is evident that REE is greater in men and women with larger body mass compared to those with smaller body sizes (Das et al. 2004; Leibel et al. 1995). Changes in body composition – increases in fat mass and decreases in FFM (Going et al. 1995) – that occur with adulthood, explain the decline in REE that has been observed in both sexes (Bemben et al. 1995; Bosy-Westphal et al. 2003; Keys et al. 1973; Poehlman et al. 1994). Moreover, studies have shown that overweight people have higher REE than their lean age-sex matched controls; this is due

to the increased body weight, and particularly FFM, to sustain the extra weight (Hoffmans et al. 1979; Ravussin et al. 1982).

The significant interaction between body size and REE has also been demonstrated after overweight and obese individuals have undergone weight reduction regimes. Most researchers agree that a reduction in REE due to weight loss is proportionate to the loss in FFM (Amatruda et al. 1993; Bessard et al. 1983; de Groot et al. 1990; Wadden et al. 1990; Welle et al. 1984). After affective weight loss regimens, REE is directly proportionate to the new reduced lean body mass (Amatruda et al. 1993; Larson et al. 1995; Nelson et al. 1992; Weinsier et al. 1995).

Individuals with low levels of REE have a significantly increased risk for weight gain (Astrup et al. 1999; Grillol et al. 2005) as opposed to those with higher REE (Ravussin 1995). However, any elevation of body weight has also been associated with compensatory changes in energy expenditure (Leibel et al. 1995). Weight gain, and hence increase in body size, increase REE (Luke et al. 2006), a phenomenon attributed, as mentioned above, to increases in FFM. For example, when patients with anorexia nervosa increase their weight during their rehabilitation, they mainly gain fat mass and this is the reason that no changes in REE occur (Onur et al. 2005). In the same group of patients, when increases in body weight are accompanied with concomitant increases in FFM, then REE is elevated (Van Wymelbeke et al. 2004).

#### 2.2.4 Exercise and Resting Energy Expenditure

There is a minority of research studies suggesting that endurance or resistance training does not affect REE (Horton and Geissler 1994; Smith et al. 1999). The majority of

studies on this subject suggest the opposite (Jamurtas et al. 2004; Osterberg and Melby 2000; Poehlman 1989; Poehlman and Melby 1998; Poehlman et al. 1988; Withers et al. 1998). The additional increase in exercise energy expenditure may be mediated by several mechanisms, including enhanced REE (Ballor and Poehlman 1992; Pratley et al. 1994; Treuth et al. 1995; Withers et al. 1998).

It is clear that energy expenditure is transiently increased due to the direct and short-term carryover effects of physical exercise (Toth and Poehlman 1996). Indeed, athletes have higher daily energy expenditure compared to sedentary individuals (McArdle et al. 2001). It is also known that habitual activity can affect REE over a long period of time even when subjects were matched for their body fat content (Poehlman 1989; Poehlman et al. 1988). This increase has a linear relationship with maximal oxygen uptake (Figure 4).

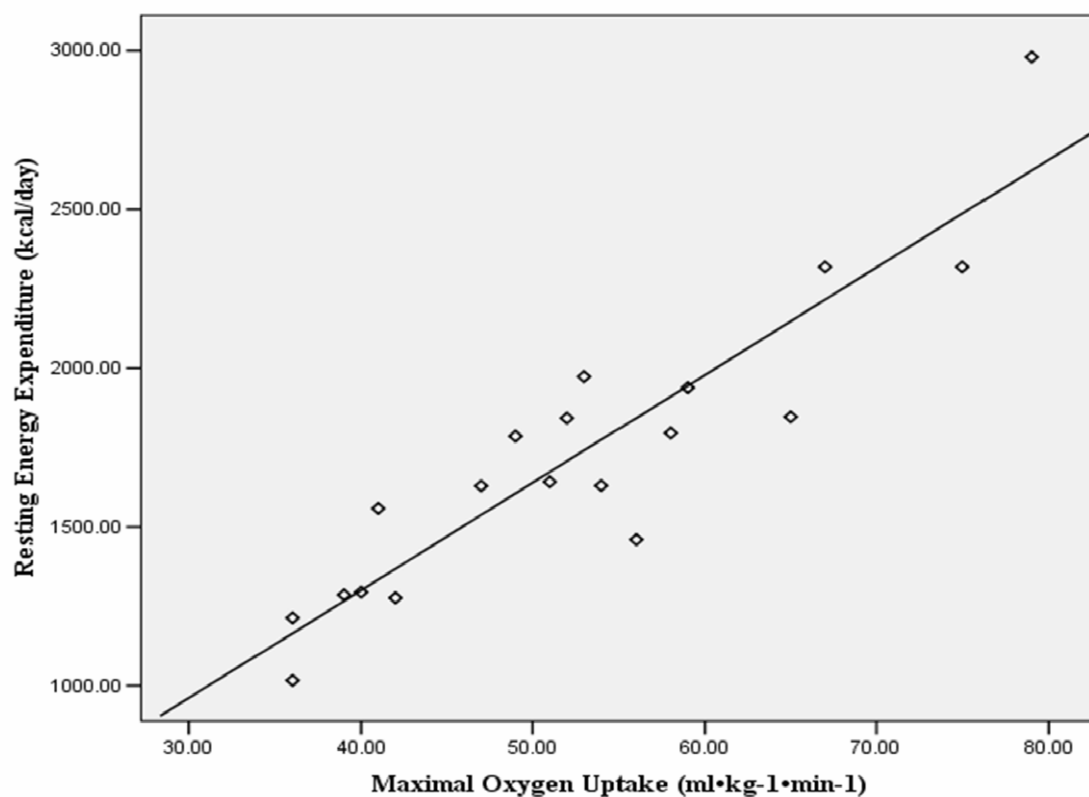
#### *Aerobic Exercise*

It has been well established that long term aerobic training as well as involvement in habitual physical activities enhances resting metabolic processes (Gilliat-Wimberly et al. 2001); this is evident despite no changes in FFM (Poehlman and Danforth 1991). The reason for this is that aerobic training results in greater REE per unit of body mass in age-matched exercisers and non-exercisers, because FFM in regular exercisers is metabolically more active (Withers et al. 1998). Moreover, aerobic training enhances REE even after a single bout of intense exercise (Jamurtas et al. 2004; Osterberg and Melby 2000).

### *Resistance Exercise*

Post-exercise REE increases after every bout of exercise (Jamurtas et al. 2004). As a result of the acute effects of strenuous exercise, REE increases due to post-exercise muscle damage, as reflected in the evaluation of total plasma creatine kinase (Schwane et al. 2000), which in turn enhances muscle catabolic and anabolic process, leading to REE augmentation (Dolezal et al. 2000). In contrast, the longitudinal changes in REE that occur with regular involvement in resistance exercise are due to increased FFM (muscle mass) as a result of normal adaptation processes (Pratley et al. 1994).

**Figure 4.** Linear relationship between maximal oxygen uptake and resting energy expenditure



### 2.2.5 Thermic Effect of Food and Resting Energy Expenditure

The thermic effect of food (TEF), otherwise known as diet induced thermogenesis, is defined as the increase in energy expenditure above basal fasting level divided by the energy content of the food ingested (Wilson and Morley 2003). Being one of the three major components of total daily energy expenditure, although the least significant, TEF has been identified as a factor that plays an important role in the regulation of body fatness (Miller and Mumford 1967) and the pathogenesis of obesity (Bessard et al. 1983; Schutz et al. 1984; Segal et al. 1985; Weststrate 1993).

Energy consumption and body weight are interrelated: the greater the amount consumed the higher the quantity of energy stored and therefore, the greater the fat content and total body weight (however, this is always depended from the amount of physical activity undertaken). For example, this is why low carbohydrate energy intake resulted in greater reductions in body weight compared to high carbohydrate diets (Wien et al. 2003). In general, different types of food (i.e. fat vs. carbohydrate vs. protein) require different amounts of energy for digestion (Westerterp et al. 1999) and also provide different amounts of energy. Moreover, the thermic effect of natural stimulants – such as caffeine (Collins et al. 1994) – also have direct and acute effects on REE

Different types of energy intake (carbohydrate vs. fat vs. protein) have different TEF. Protein and carbohydrate ingestion have a relatively larger calorogenic effect compared to fat, thus a higher thermic effect (Westerterp-Plantenga et al. 1999). Relevant studies have shown that *ad libitum* consumption of high protein/low fat averages less kcal/day compared to a high carbohydrate/low fat diet (Skov et al. 1999). Moreover, studies have also shown that individuals consume less energy (about 20%) when given

high protein meals vs. high carbohydrate meals (Araya et al. 2000; Barkeling et al. 1990; Latner and Schwartz 1999). The reason for these differences is the impact that protein ingestion has on satiety. In particular, proteins, unlike fats, starches or glucose, are potent stimulators of cholecystokinin, the major gastrointestinal hormone inducing satiety (Liddle et al. 1986). As such, when energy intake is matched and dietary fat is restricted to less than 30% of the meal, the replacement of a moderate percentage of energy from carbohydrate and/or protein (i.e., 15% of energy) has been shown to enhance weight loss (Baba et al. 1999; Layman et al. 2003) and fat loss (Baba et al. 1999; Parker et al. 2002) while maintaining FFM (Farnsworth et al. 2003; Piatti et al. 1994; Vazquez et al. 1995).

Modest energy restriction significantly lowers REE, but relative to comparable high carbohydrate diets, hypoenergetic high protein diets appears to spare REE. In obese subjects adhering to a reduced-energy high carbohydrate diet for four weeks, REE fell by 17% whereas, in subjects adhering to a reduced-energy high protein diet, REE fell only by 6% (Baba et al. 1999). The difference in REE as a result of a diet with high protein appears as early as six days after the initiation of the energy restriction regime and may relate to improved nitrogen balance (Agus et al. 2000). In general, the thermic response to protein ingestion is 50% to 100% higher than that for carbohydrate (Schutz et al. 1987; Zed and James 1986), an effect mainly attributed to the metabolic costs of protein synthesis, ureogenesis and gluconeogenesis. It has been estimated that the difference in diet-induced thermogenesis between a combined high protein / high carbohydrate diet vs. high-fat, low protein/carbohydrate diet amounted to an extra 90 kcal over a 24-hour period (Westerterp et al. 1999).



### 2.2.6 Stress and Resting Energy Expenditure

Stress is a term for a wide range of external stimuli, both physiological and psychological. It is often assumed that psychological stress is a stressful stimulus; however, it has been shown that it does not induce alterations in REE (Weststrate et al. 1990). Stressful stimuli that alter energy expenditure include the exposure to either cold or hot ambient temperatures (van Marken Lichtenbelt et al. 2002; van Marken Lichtenbelt et al. 2001). For example, resting metabolism is significantly increased following cold exposure, as shivering generates body heat for the maintenance of a stable core temperature (van Marken Lichtenbelt et al. 2002); this, in turn, increases REE up to five-fold compared to normal environmental conditions (McArdle et al. 2001). This is the reason why assessment of REE has to be performed in a thermo-neutral room.

#### *Smoking*

Active and passive smoking also represent two external stimuli that profoundly alter resting metabolism (Metsios et al. 2007; Walker and Kane 2002). The altered metabolism is attributed to significant dose-dependant increases in thyroid hormones (Fisher et al. 1997). Moreover, smoking has been associated with an imbalance in the production of tumour necrosis factor alpha [(TNF $\alpha$ ), pro-inflammatory cytokine involved in the biochemical processes of muscle wastage and atherosclerosis] and soluble TNF receptors, leading to a relative excess of TNF $\alpha$  (Glossop et al. 2006). Through the increased REE, chronic smoking or exposure to passive smoking may significantly alter body composition leading to enhanced loss of FFM rather than fat mass (Akbarbartoori et al. 2005; Metsios et al. 2007). It seems, therefore, reasonable to suggest that smoking

in combination with chronic diseases will have detrimental health effects. However, no studies to date have investigated this relationship (smoking + chronic disease) on REE.

#### 2.2.7 Disease and Resting Energy Expenditure

Several lines of evidence suggest that disease states and their treatment may alter metabolism (Batterham 2005; Daneryd et al. 1998; Schols et al. 1991; Utaka et al. 2005; Walsmith and Roubenoff 2002; Wang et al. 2004). In conditions such as human immunodeficiency virus infection, cancer and rheumatoid arthritis, changes in metabolism have been attributed to the excess production of relevant pro-inflammatory cytokines (Argiles et al. 2006b; Rall and Roubenoff 2004). These processes enhance protein catabolism resulting in muscle wasting and thus, reduced FFM. In conditions, e.g. anorexia or obesity, alterations in metabolism are directly linked with changes in body composition (Obarzanek et al. 1994). In general, there are several diseases that have been investigated in relation to REE, as altered metabolism may lead to malnutrition and cachexia, both leading to premature mortality (Miles 2006).

##### 2.2.7.1 Human Immunodeficiency Virus (HIV) Infection

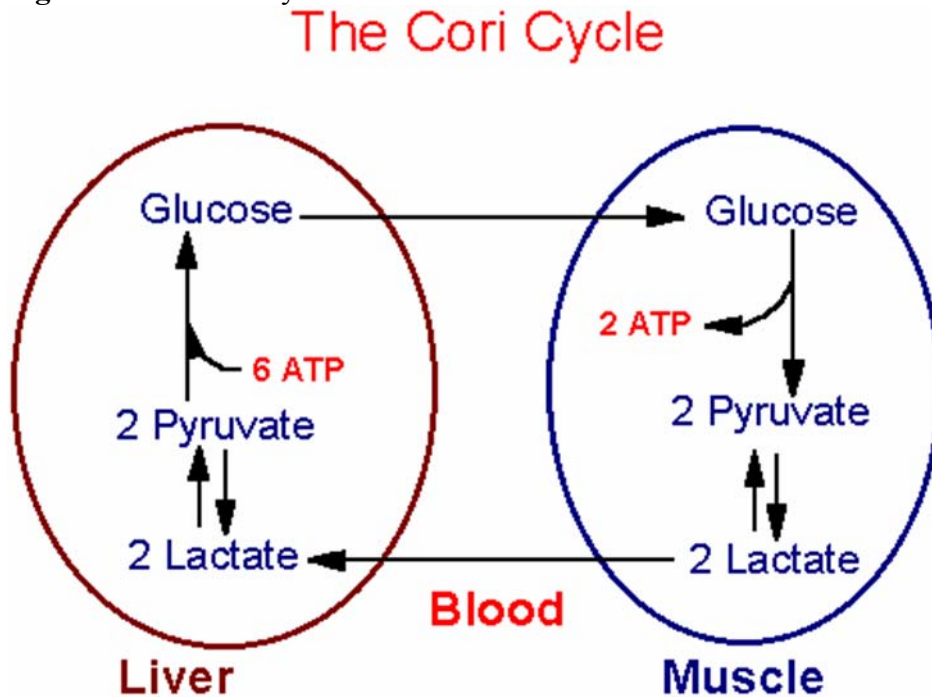
Some studies suggest that HIV infection is accompanied by cytokine-driven increases in REE, particularly during symptomatic disease (Suttman et al. 2000) with weight loss, which is predominantly due to loss of FFM (Roubenoff et al. 2002). However, other studies have failed to demonstrate any interaction between REE and cytokines in HIV patients (Godfried et al. 1995). Difficulties in measuring cytokine activity (Hellerstein et

al. 1996; Roubenoff et al. 2002) may have accounted for such discrepancies. Nevertheless, a recent meta-analysis has demonstrated that HIV infected patients experience elevated REE (hypermetabolism) compared to the normal population (Batterham 2005). Treatment with highly active antiretroviral therapy does not suppress this elevation in REE (Shevitz et al. 1999).

#### 2.2.7.2 Cancer

It has been well documented that cancer is associated with increased levels of REE (Falconer et al. 1994; Staal-van den Brekel et al. 1995; Van Cutsem and Arends 2005). One of the most important clinical manifestations of cancer is the cachexia-anorexia syndrome. In tumour-bearing states, cachectic factors such as cytokines elicit effects on energy homeostasis; the increased hypothalamic actions of these mediators induce anorexia and unopposed weight loss (Inui 2002). Several cytokines have been proposed as mediators of this cachectic process, among which are TNF $\alpha$  and interleukins (Baracos 2000; Moldawer and Copeland 1997). Specifically, TNF $\alpha$  significantly affects muscle repair processes (Tisdale 2000) via the activation of the proteasome system and transcription factor NF-kb leading to decreased expression of MyoD, which is important for replenishing wasted muscle (Guttridge et al. 2000). Cytokines also delay gastric emptying, lower serum albumin concentrations, and enhance lipolysis (Davis et al. 2004; Ramos et al. 2004). In addition, most solid tumors produce large amounts of lactate, which is converted back into glucose in the liver by the Cori cycle [(Tisdale 2000), (Figure 5)].

**Figure 5.** The Cori Cycle.



Gluconeogenesis from lactate uses ATP and is very energy inefficient for the host. This futile cycle may be responsible for the increased energy expenditure. A 40% increase in hepatic glucose production has been reported in weight-losing cancer patients, which may also be a consequence of meeting the metabolic demands of the tumour and therefore, it contributes to the development of the cachectic process (Baracos 2000; Inui 2002). All these processes are responsible for the increase in resting metabolism and cause cachexia (Mullen 1994).

#### 2.2.7.3 Rheumatoid Arthritis

RA has been directly linked with increased levels of resting metabolism (Metsios et al. 2006; Rall and Roubenoff 2004), a metabolic abnormality leading to rheumatoid

cachexia. In brief, REE elevation in the RA population is partly a result of cytokine overproduction, particularly TNF $\alpha$  (Roubenoff et al. 1994). The causes and consequences of this metabolic alteration in RA are described in the next section of the current chapter under the heading Rheumatoid Cachexia.

#### 2.2.7.4 Cachexia-Anorexia Syndrome

The cachexia-anorexia syndrome occurs in chronic pathophysiologic processes including HIV infection (Berenstein and Ortiz 2005), cancer (Inui 2002), liver disease (Laviano et al. 2005), obstructive pulmonary disease (Femia and Goyette 2005), and rheumatoid arthritis (Walsmith and Roubenoff 2002). Cachexia makes an organism susceptible to secondary pathologies and can result in death. The main causes of this multifactorial syndrome are pain, depression or anxiety, changes in taste perception and food aversions, vomiting, malfunction of the gastrointestinal system, metabolic alterations, and cytokine overproduction (Femia and Goyette 2005; Laviano et al. 2005).

The cachexia-anorexia syndrome also involves metabolic and immune changes mediated by such the pathophysiologic process (i.e., tumour or host-derived cytokines) and is associated with lipolysis and acceleration of proteolysis which, in turn, result in the loss of fat mass and FFM, respectively (Brown et al. 2003). As a result, REE increases dramatically in cachectic patients in parallel with rapid weight loss, indicating a systemic dysregulation of metabolism (Plata-Salaman 2000). Moreover, anorexic patients have higher rates of REE due to increased protein turnover, a phenomenon that diminishes during refeeding (Winter et al. 2005).

#### 2.2.7.5 Other diseases

REE has been investigated in relation to various other diseases, mainly to optimize nutritional strategies in order to avoid anorexia, malnutrition and cachexia. Such diseases include sickle cell anaemia (Buchowski et al. 2002), renal failure (Scheinkestel et al. 2003), sepsis (Uehara et al. 1999), severe burns (Suman et al. 2006), juvenile rheumatoid arthritis (Knops et al. 1999) and anorexia nervosa (Winter et al. 2005). In general, in critical illness it is necessary to accurately estimate REE, given that patients receive nutritional support, to avoid complications associated with under- and overfeeding (Flancbaum et al. 1999; Ogawa et al. 1998). For this reason, in such critical conditions, for example in mechanically ventilated patients, REE should preferably be assessed accurately via the use of indirect calorimetry rather than using existing prediction REE formulae (Alexander et al. 2004; Vazquez Martinez et al. 2004).

### 2.3 Evaluation of Resting Energy Expenditure

#### 2.3.1 Indirect Calorimetry

Indirect calorimetry is the main assessment for the evaluation of REE, as it represents accurate and clinically feasible means of measuring energy expenditure. It is “indirect” because the caloric burn rate is calculated from the evaluation of oxygen uptake. In contrast, direct calorimetry implies a measurement of heat released by the body, which is technically difficult and clinically impractical. For this reason, indirect calorimetry is the

most widely used method of determining the REE; it estimates REE by converting oxygen uptake into an estimate of resting metabolism (Scott et al. 2006).

The explanation of using oxygen uptake for determining energy demands is that the body stores little oxygen at rest, while this amount is significantly increased during maximal and sub-maximal exercise. Therefore, the amount of oxygen that enters the lungs and then the blood is directly proportionate to the amount used by the tissues for oxidative metabolism. Hence, an accurate estimate of energy production can be predicted by measuring the oxygen uptake. Similarly, oxygen and carbon dioxide exchanged in the lungs equal that used and released by the tissues. With this knowledge, caloric expenditure can be calculated by measuring the respiratory gases. The calculation of oxygen consumption is possible after establishing the volume of air inspired and expired ( $V_I$  and  $V_E$ ) as well as the fractions of oxygen in these volumes ( $F_{IO_2}$  and  $F_{EO_2}$ ) in a given time:

$$VO_2 = (V_I \times F_{IO_2}) - (V_E \times F_{EO_2})$$

Since the early 1980's, the application of indirect calorimetry for determining oxygen consumption and carbon dioxide production through metabolic measurements has continued to increase. The accuracy of devices and methods used to obtain this information has greatly improved over the years (Makita et al., 1990; McClellan et al., 1999). From analyzing expiratory gases with mass spectrometry, to the more recent technology of mixing chambers, metabolic monitors have evolved to small bedside modules ensuring measurement accuracy for both healthy and diseased populations, including patients with cancer (Faulding et al., 2005), diabetes (Nowata et al., 2004),

HIV (Kaminski et al., 2003), and chronic inflammatory diseases (Roubenoff et al., 1994). The accuracy of individual indirect calorimetry systems from a very wide range – including whole-room calorimeters, doubly labelled water, open-circuit Douglas bag methods, metabolic carts, ventilated-hood systems, and a handheld device (i.e. the SenseWear system armband which collects information for movement, heat flux, skin temperature and sweating) – has been established and is well known for each system (Conway et al. 2002; King et al. 1999; Nieman et al. 2003; Phang et al. 1990; Sun and Hill 1993; Tissot et al. 1995).

Although considered as the gold standard for the assessment of REE, indirect calorimetry has disadvantages. As a method, it needs trained personnel, it is time consuming, and requires expensive and specialized equipment. In addition, indirect calorimetry software cards [those not using the Weir equation (Weir 1990) for the prediction of REE] could be considered inappropriate in special circumstances such as in critically ill patients (Makk et al. 1990; McClave and Snider 1992).

#### *Variation in the assessment of Resting Energy Expenditure*

Studies investigating day-to-day variability in REE have highlighted that many factors – including diurnal variation, TEF, elevated post-exercise oxygen consumption, stimulants, and pharmaceuticals – may affect metabolic rate (Melby et al. 1993; Reed and Hill 1996; Shannon et al. 1999). If these factors are controlled for, bias is reduced. Morning and evening REE measurements are very highly correlated, although the evening measurement (mainly due to TEF) is ~100 kcal/d higher which is found to be non-significant (Haugen et al. 2003). Previous and recent studies have reported that within-subject, day-to-day variation of REE ranges from 2% to 10% (Black and Cole 2000; De



Boer et al. 1987; Gibbons et al. 2004; Rumpler et al. 1990; Toubro et al. 1995; Ventham and Reilly 1999).

### 2.3.2 Prediction Equations

The above mentioned disadvantages of indirect calorimetry led to the development of prediction formulae, as an alternative way to calculate REE. These equations have been mainly developed from data obtained from normal healthy individuals (Table 1); however, given the significance of resting metabolism, REE equations have also been developed for some diseased populations (de Luis et al. 2006; Martin et al. 2004; Williams et al. 2002). A summary of available formulae appears in Table 1. Although easy to use, inexpensive, and universally available, these equations have been found to be inaccurate within certain clinical settings and vary considerably from the gold standard values obtained by REE measurements using indirect calorimetry methods (Bauer et al. 2004; Dickerson et al. 2002; Zauner et al. 2006).

<b>Table 1.</b> Resting energy expenditure prediction equations	
Author	Equation
Harris-Benedict (Harris and Benndict 1919)	Males: $(13.75 \times W) + (5.3 \times H) - (6.78 \times A) + 66.5$ Females: $(9.56 \times W) + (1.85 \times H) - (4.68 \times A) + 655.1$
Owen (Owen et al. 1987; Owen et al. 1986)	Males: $(10.2 \times W) + 875$ Females: $(7.18 \times W) + 795$
Mifflin (Mifflin et al. 1990)	Males: $(10 \times W) + (6.25 \times H) + (5 \times A) + 5$ Females: $(10 \times W) + (6.25 \times H) + (5 \times A) - 161$
20kcal/kg ratio (ASPEN 2002b)	All subjects: $W \times 20$
Cunningham (Cunningham 1991)	All subjects: $21.6 \times \text{FFM} + 370$
Wang (Wang et al. 2000)	All subjects: $21.5 \times \text{FFM} + 407$
Bioelectrical Impedance	Based on FFM but not given in the manual
Luke-Sheller (Luke and Schoeller 1992)	All subjects: $20 \times \text{FFM} + 585$
Revising (Ravussin et al. 1982)	All subjects: $20.82 \times \text{FFM} + 471$
Revising (Ravussin et al. 1986)	All subjects: $20.93 \times \text{FFM} + 478.7$
Elia (Elia 1992)	All subjects: $21.11 \times \text{FFM} + 450$
McNeill (McNeill et al. 1987)	All subjects: $21.5 \times \text{FFM} + 329$
Heymsfield (Heymsfield et al. 1988)	All subjects: $21.6 \times \text{FFM} + 302$
Kasiwazachi (Kasiwazaki et al. 1988)	All subjects: $24.5 \times \text{FFM} + 304$
Where W=weight in kg; H=height in cm; A= age in years and FFM=fat-free mass	

As shown in Table 1, the factors that are commonly used for REE prediction are either anthropometrical characteristics (weight, height and age) or FFM. In the normal population, both factors reveal a reasonable accuracy in the REE prediction (Muller et al. 2004). It has been suggested that FFM is a better predictor in adolescents because it is the only biologically active metabolic component (Wang et al. 2000) and therefore the major determinant of REE prediction (Cahill 1972; Muller et al. 2004).

## 2.4 Rheumatoid Arthritis

### 2.4.1 Definition

RA is a chronic symmetrical inflammatory polyarthritis. It is accompanied by unpredictable periods of exacerbation (flares) and remission and can result in disability, joint destruction due to bone erosion, reduced range of movement, fluctuating pain and physiological distress (Pincus and Callahan 1993).

### 2.4.2 Aetiology and Pathogenesis

With an estimated prevalence of 0.5 – 1% in Europe and North America [(currently 0.8% of the adult population in the UK), (Symmons et al. 2002)] RA is the commonest chronic inflammatory joint disease. Although the exact pathogenesis of the disease remains unknown, several factors have been linked with its development. These include the following:

#### *Genetic Susceptibility*

It is postulated that a genetically susceptible host is exposed to an unknown antigen and that this interaction gives rise to a persistent immunological response. The fact that first degree relatives of individuals with RA develop the disease in a higher rate than the general population, connotes a genetic predisposition to RA (Deighton et al. 1992). The most definite genetic association with RA are specific antigens within the major histocompatibility class II complex (Human Leucocyte Antigen-HLA DRB1\*0404 and 0401).

### *Gender*

RA affects predominantly women (female to male ratio: 2 – 3.1) and this implies that sex hormones may affect the development of the disease. This is reinforced by the fact that pregnancy has an ameliorating effect on RA and that RA patients are more likely to be nulliparous before disease onset than controls (Hazes 1991). The presence of alloantibodies in the maternal circulation developed against paternal HLA, may be a possible explanation (Combe et al. 1985). Another possible explanation for sex hormones and RA is the changes in the hormonal profile. Estrogens and progesterone are implicated in the immune response as enhancers of the humoral (Th2) and inhibitors of cellular (Th1) immune responses (Cutolo and Lahita 2005). RA synovitis is mainly driven via Th1-type of immune responses. The oral contraceptive pill may protect from RA or associate with a milder disease course (van Zeben et al. 1990; Wingrave and Kay 1978).

### *Infectious Agents*

Viruses [Epstein-Barr (Sawada and Takei 2005); parvovirus (Caliskan et al. 2005); lentiviruses (Wilder 1994)] and bacteria [mycoplasma (Silman and Pearson 2002); mycobacteria (van der Heijden et al. 2000); yersinia (Koehler et al. 1998) amongst others] have been proposed as possible agents contributing to the pathogenesis of RA but evidence of their involvement is still lacking. These organisms may infect host cells and thus trigger a T-cell mediated immune response, which can be perpetuated through yet-to-be determined mechanisms.

### 2.4.3 Disease Characteristics

RA can affect every joint where cartilage overlies bone and with a joint cavity lined by synovial membrane and containing synovial fluid (Figure 6). The major site of the disease is the synovium. During the early stages of the disease, the most noticeable characteristic is tissue oedema which is clinically manifested with pain and swelling (Jansen et al. 2001). Vessel proliferation and angiogenesis are also noticeable in arthroscopy, and synovial lining hyperplasia starts to develop (Koch 2000). Hyperplasia becomes more pronounced as the disease progresses (Henderson et al. 1988). The most obvious manifestation of RA as the disease becomes chronic, is the exuberant synovial infiltration by mononuclear cells, consisting of T cells, B cells, macrophages and mature B lymphocytes. Angiogenesis continues and both the degree and content of the cellular infiltration changes (Koch 2000). A hyperplastic, aggressively invasive tissue develops in the joint margin ("panus" tissue), which is full of inflammatory cells, producing a range of oxygen free radicals and proteolytic enzymes, which may cause permanent cartilage and erosive bone damage (Tak 2001).

**Figure 6.** Differences between a normal joint and a joint affected by rheumatoid arthritis.

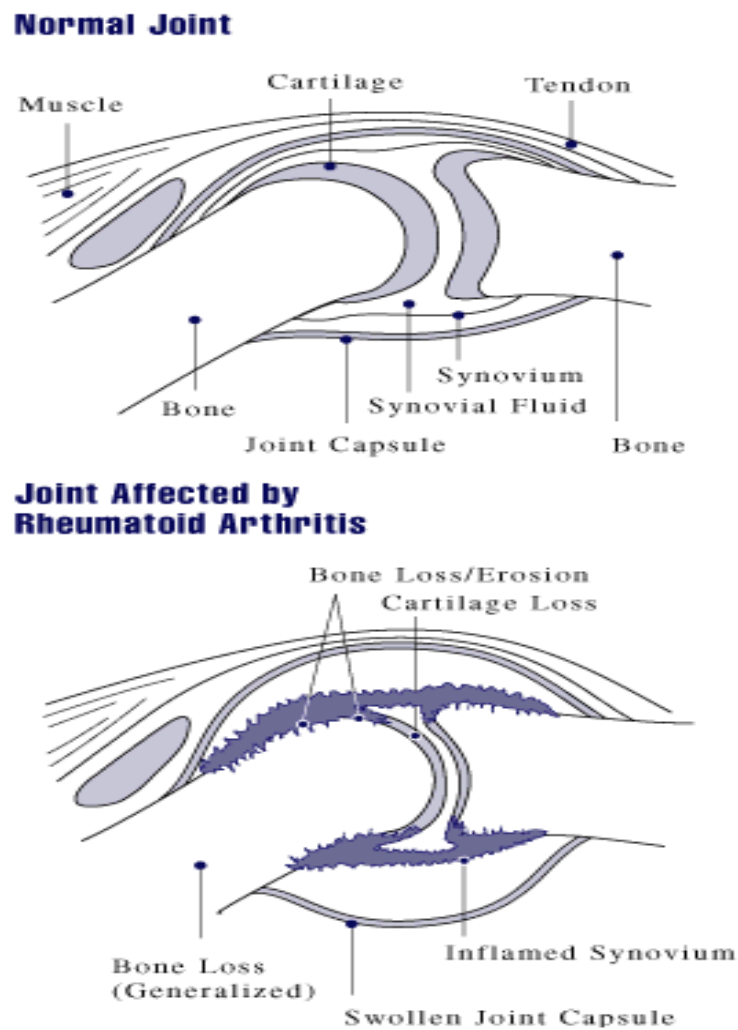


Figure adopted from: [http://www.pueblo.gsa.gov/cic\\_text/health/rheumatoid/Joint\\_vert.gif](http://www.pueblo.gsa.gov/cic_text/health/rheumatoid/Joint_vert.gif)

#### 2.4.4 The Role of Cytokines

Cytokines were originally characterised as low-molecular weight proteins or glycoproteins (proteins which contain carbohydrate) that are secreted by cells involved in the immune response. They function as messengers of the immune response by providing communication among macrophages and lymphocytes. During an immune response

cytokines bind to specific receptors on a neighbouring cell, and instruct that target cell to respond in a genetically programmed manner (Alcocer-Varela 2001). After cytokine stimulation, a target cell may respond in many ways. A common response is an increased production of proteins that are inserted in the plasma membrane and function as receptors. Many of these receptors are specific for various cytokines, but others are necessary for protection against infectious agents. In addition, most cytokines will also cause the target cell to initiate proliferation and differentiation. Because this effect is common among cytokines, there is a great deal of redundancy, in that different cytokines may have similar effects on the same target.

The physiological role of cytokines concerns homeostasis, the control of development of leucocyte lineages, the activation of inflammatory mechanisms and the repair and remodelling of damaged tissue. It has also been established that cytokines act as mediators of pathology in infectious, inflammatory and immune diseases. When inflammation occurs in the synovial tissue, it causes release of enzymes, growth factors, and cytokines. The later activate directly fibroblasts, chondrocytes and osteoclasts in the articular cartilage surface thus, leading to the release of destructive enzymes and the inhibition of matrix synthesis (Miossec 1992).

Cytokines can be classified into groups according to their origin and function in inflammation. In the rheumatoid joints, a variety of cytokines are produced locally. The two prototypic pro-inflammatory cytokines are interleukin 1 (IL-1) and TNF $\alpha$  and have a significant effect in the pathophysiological mechanisms causing synovial inflammation and progression of joint destruction (Duff 1994; Gabay 2002). Table 2 depicts the main source and function of selected pro-inflammatory cytokines.

**Table 2.** Source and function of specific pro-inflammatory cytokines

<i>Cytokines</i>	<i>Source</i>	<i>Function</i>
Tumour Necrosis Factor alpha	Macrophages, T cells, B cells, NK cells, mast cells	Increases cytokine production; increases inflammatory and immune responses.
Interleukin 1 (a and b)	Predominantly macrophages	Increases immune response; inflammatory mediator; activates T cells; activates phagocytes; increases prostaglandin production, induces fever.
Interleukin 6	Monocytes, T (Th2) and helper T cells, macrophages	B cell stimulatory and differentiation factor; increases hematopoiesis; increases inflammatory response; increases fever.

The exact biochemical processes which stimulate the overproduction of IL-1 and TNF $\alpha$  in the rheumatoid joint, remain unclear; however, the main source may possibly be the macrophages in the inflamed synovium. On cell surfaces, cytokines bind with specific receptors resulting in stimulation of signal transduction pathways that lead to increased or decreased gene transcription. The most important signal transduction pathways in the rheumatoid synovium are: a) AP-1 pathway and b) NF-kB pathway (Firestein and Manning 1999). The latter has been found to be particularly important in chronic inflammatory diseases, mediating the production of IL-1 and TNF $\alpha$  but also interceding their effects on target cells after they have bound to cell surface receptors. Stimulation of these signal transduction pathways leads to the release of collagenases and other enzymes from target cells, other pro-inflammatory molecules and more cytokines, that contribute to the joint destruction (Arend 2001).



There is a general agreement that both IL-1 and TNF $\alpha$  significantly contribute in the pathogenesis of RA. IL-1 is more responsible for the destructive aspect of the disease (Jacques et al. 2006), whereas TNF $\alpha$  accounts for the proliferative and inflammatory aspects of the disease (Hasan 2006). IL-1 and TNF $\alpha$  act in a synergistic manner, having both local and systemic effects on the disease.

#### *Local effect of cytokines*

Locally, IL-1 and TNF $\alpha$  up-regulate the expression of adhesion molecules (i.e. sICAM-1, E-Selectin) on endothelial cells in synovial post-capillary venules, enhancing the migration of leucocytes from circulation into the inflamed joints (Mojcik and Shevach 1997). They also contribute in the growth of new blood vessels, which also characterizes the rheumatoid synovium (Arend 2001). Most importantly, IL-1 and TNF $\alpha$  are the key mediators of tissue destruction in RA joints through inducing the synthesis and release of enzymes from synovial fibroblasts and articular chondrocytes. These cytokines further contribute to local inflammation by inducing the production of reactive oxygen intermediates, reactive nitrogen intermediates, and prostaglandins (Darlington and Stone 2001).

Elevated levels of interleukin 6 (IL-6), stimulated by IL-1 and TNF $\alpha$ , have also been found in the synovial fluid and serum in RA patients compared to age-matched controls (Kotake et al. 1996). IL-6 modulates the balance between three metalloproteinases (pro-matrix metalloproteinases 1, 3, and tissue inhibitor of metalloproteinase) at sites of inflammation in RA. Alteration in this balance leads to joint and cartilage destruction (Wong et al. 2000).

### *Systemic effects of cytokines*

RA is also characterized by systemic involvement. Hence, apart from the local effects of cytokines, several organs and biological procedures are altered in response to the disease.

The body reacts to inflammation with the acute phase response. This is a generalized response when physiological homeostasis is disturbed. The responsible mediators are cytokines, and the liver is their predominant target organ in this context (Gabay 2006). Normally, the acute phase response lasts only a few days; however, in cases of chronic or recurring inflammation such as in RA, an aberrant continuation of some aspects of the acute phase response may contribute to the underlying tissue damage that accompanies the disease, and may also lead to further complications (e.g., cardiovascular disease). In the hepatic acute phase response,  $\text{TNF}\alpha$ , IL-1 and IL-6 play a key role (Heinrich et al. 1998; Heinrich et al. 1990; Ingenbleek and Young 1994; Le and Vilcek 1989). They activate hepatocyte receptors, and synthesis of varying acute phase proteins starts. IL-6 is the major signal for secretion of most of the acute phase proteins by hepatocytes (Heinrich et al. 1998; Le and Vilcek 1989). Furthermore,  $\text{TNF}\alpha$  causes muscle catabolism that is also mediated by glucocorticoids, as well as glucagon-induced hyperglycaemia and amino acid uptake by the liver. IL-1 stimulates an increase in whole body amino-acid flux, and activation of the pituitary-adrenal axis. It has been shown that Kupffer cells also play an intermediate role (Knolle et al. 1995). After stimulation by the pro-inflammatory cytokines, Kupffer cells form IL-6 and present it to the hepatocytes. IL-6 depresses mononuclear phagocytic production of IL-1 and  $\text{TNF}\alpha$  (Schindler et al. 1990) thus mitigating the whole cascade reaction. Down-regulation of the hepatocytic acute phase response is achieved by rapid hepatic removal of circulating cytokines

(Heinrich et al. 1998), release of interleukin-10 by the Kupffer cells which results in suppression of the local IL-6 production (Knolle et al. 1995).

In the acute phase response, several acute phase reactants are overproduced, having both positive and negative impact. One of the major positive acute phase reactants is C-reactive protein (CRP), which has a remarkable sensitivity, speed and dynamic range of responses. The average serum concentration of CRP is 0.8 mg/L (Shine et al. 1981) but following an acute-phase stimulus, values may increased by as much as 10.000-fold, with the synthesis of CRP starting very rapidly, serum concentrations beginning to rise by about six hours, and peaking around 48 hours after a single stimulus (Kushner et al. 1978). The levels of CRP slightly elevate with age, while females have slightly higher circulating concentrations (Hutchinson et al. 2000). In many diseases, the circulating value of CRP reflects accurately on-going inflammation compared to other biochemical parameters of inflammation, such as the erythrocyte sedimentation rate. It has been well established that RA is accompanied by elevated levels of CRP due to the systemic involvement of the disease (Kristensen et al. 2006; Zink et al. 2005). Moreover, compelling evidence in the literature suggests that CRP is a sensitive marker for the development of cardiovascular disease, as it contributes to the development of atherosclerosis (Labarrere and Zaloga 2004; Li and Fang 2004) via several biochemical mechanisms (e.g., by damaging arterial endothelium and promoting the development of atherosclerotic lesions). As such, CRP is now considered as a risk factor for the development of cardiovascular disease (de Ferranti and Rifai 2007). For these reasons evaluation of the levels of CRP are of high prognostic and preventive significance (Pepys and Hirschfield 2003).

#### 2.4.5 Oxidative Stress and Rheumatoid Arthritis.

Free radicals are capable of damaging cellular components such as lipids, proteins and DNA, while accumulating evidence suggests that they also contribute to various disease entities including inflammatory joint disease. There is now much direct and indirect evidence that free radicals are implicated in the development of inflammatory arthritis. Cells present in inflamed joints (e.g., macrophages, neutrophils and lymphocytes), have the ability, when isolated and stimulated, to produce free radicals (Maly et al. 1988). There is also evidence directly linking free radicals with articular damage, which is one of the main manifestations of RA. The mechanism for articular damage is that hydroxyl radical can degrade cartilage, hypochlorous acid can attack proteoglycans (integral components of structural tissues) whereas hydrogen peroxide can inhibit proteoglycan synthesis (Hadjigogos 2003); these processes of degradation and inhibition could facilitate the damage of the cartilage.

In patients with inflammatory arthritis there are abnormalities consistent with oxidative damage. The serum and synovial fluid contain end products of lipid peroxidation which have been found to correlate with disease severity and activity (Rowley et al. 1984). Moreover, hydrogen peroxide stimulates the upregulation of NF- $\kappa$ B signaling pathway, which is responsible for joint destruction since it enhances the production and activity of pro-inflammatory cytokines (Winrow et al. 1993). However, the upregulation of signaling through the NF- $\kappa$ B pathway has also been implicated in protein degradation which leads to loss of FFM in RA (Lecker et al. 1999). As such, free radical production may also be involved in the processes that cause rheumatoid cachexia.

## 2.5 Pharmacological Approaches in Rheumatoid Arthritis

There is no known cure for RA. To date, the goal of treatment in rheumatoid arthritis is to reduce joint inflammation and pain, maximize joint function, and reduce or prevent joint destruction and deformity. Early medical intervention has been shown to be essential in improving outcomes (Huizinga and Landewe 2005). Early aggressive management can improve function, stop damage to joints as seen on x-rays, and prevent work disability (Chen and Wei 2005). In addition to pharmacological interventions, optimal treatment of the disease involves a combination of medications, rest, joint strengthening exercises, joint protection, and patient (and family) education, while treatment is customized according to many factors.

### 2.5.1 First and Second Line Drugs

Non-steroidal anti-inflammatory drugs, corticosteroid medications, and disease-modifying anti-rheumatic drugs are medicines administered in RA patients to reduce tissue inflammation, pain, swelling, and improve the overall disease status. The physiological mechanisms of each medication are different while their administration depends on the severity of concurrent RA status.

#### *Non-steroidal anti-inflammatory drugs (NSAIDs)*

These are medications that do not contain cortisone. Their therapeutic purpose is to minimize day-to-day inflammation, which can permanently damage cartilage and bone over a short period of time. Persistent inflammation can usually be reduced by NSAID therapy within two to four weeks of continuous use (ARC 2002). In general, this is very

large category of medications - which range from the familiar aspirin to ibuprofen (Motrin) as well as to the newest class of NSAIDs, the coxibs. The main action of all NSAIDs is to block the enzyme called cyclooxygenase; this in turn enables the development of prostaglandins which trigger inflammation (Wood 1999).

### *Corticosteroids*

Corticosteroids are medication that contain cortisone. Cortisone injections and prednisone are often used to treat a range of chronic autoimmune inflammatory disease including RA. Corticosteroids reduce inflammation by decreasing the action of the body's immune response. While this effect can help relieve pain and swelling, it makes patients more susceptible to infection. Use of corticosteroids in low doses has been found to be effective in reducing inflammation caused by rheumatoid arthritis (Emery et al. 2002). A recent study showed that two years of continuous low-dose prednisolone therapy slowed the progression of joint damage (Pisetsky and St Clair 2001). Corticosteroid injections into inflamed joints can relieve pain and increase functional ability, which may last from weeks to months. However, one of the most common side effects of this treatment is the enhancement of collagen degradation (Cohen et al. 1977), the protein that is the main support of skin, tendon, bone, cartilage and connective tissue..

### *Disease-modifying anti-rheumatic drugs (DMARDs)*

Persistent inflammation in several joints due to inflammatory arthritis for longer than six weeks requires stronger medicine, which is when DMARDs (also known as second-line agents and slow-acting anti-rheumatic drugs) have to be prescribed (ARC 2002). Rheumatologists usually prescribe this class of medication in addition to anti-inflammatory NSAIDs. While the NSAID reduces day-to-day inflammation, the DMARD slows down the biological processes that drive persistent inflammation. The

most commonly used DMARDs are methotrexate, gold therapy, sulfasalazine, and azathioprine.

### 2.5.2 Newer Treatments

The pro-inflammatory effect of TNF $\alpha$  suggests that inhibition of TNF $\alpha$  would be clinically useful in RA. Indeed, extensive data from clinical trials have confirmed the efficacy of all three currently available TNF inhibitors in relieving the signs and symptoms of RA, and in slowing or halting radiographic damage (Edwards 2005; Finckh et al. 2006; Puppo et al. 2005). However, the long-term safety of these agents is still relatively unknown.

#### *Etanercept (Enbrel)*

Etanercept is a human fusion protein that combines two extracellular binding domains of the p75 form of the TNF $\alpha$  receptor to the Fc portion of a human IgG1 antibody molecule. The protein is entirely human and anti-etanercept antibodies are relatively uncommon. In clinical trials, etanercept proved to be safe and effective in reducing the signs and symptoms of RA, as well as in slowing / stopping radiographic damage, when used either as monotherapy or in combination with methotrexate (Farahani et al. 2006; Haraoui 2005; van der Heijde et al. 2006). *Mechanism:* Etanercept binds TNF $\alpha$  in the circulation and in the joint, preventing interaction with cell surface TNF $\alpha$  receptors thereby reducing TNF $\alpha$  activity (Keating and Perry 2002).

#### *Infliximab (Remicade)*

Infliximab is a chimeric monoclonal antibody that binds TNF $\alpha$  with high affinity and specificity. The antibody binding site for TNF $\alpha$  is of mouse origin, with the remaining

75% of the infliximab antibody derived from a human IgG1k antibody sequence. Infliximab has been studied extensively in clinical trials. It is effective as monotherapy in reducing the signs and symptoms of RA. Co-treatment with methotrexate reduces the frequency of anti-infliximab antibodies and is therefore recommended along with infliximab. The combination of infliximab and methotrexate is very effective in reducing clinical manifestations of the disease, as well as in halting radiographic progression of disease in RA (Geletka and St Clair 2005; Smolen et al. 2006; Westhovens et al. 2006).

*Mechanism:* Infliximab binds TNF $\alpha$  in the joint and in the circulation, preventing its interaction with TNF $\alpha$  receptors on the surface of inflammatory cells, and eventually clearing TNF $\alpha$  from the circulation (Calabrese 2003).

#### *Adalimumab (Humira)*

Adalimumab is a recently approved anti-TNF $\alpha$  medication that differs from infliximab in that its sequences are entirely human. Generated by phase display technology with amino acid sequences only from the human germline, it is indistinguishable in structure and function from natural human IgG1. Adalimumab also has high specificity for TNF $\alpha$  and a half-life of approximately two weeks. Like the other TNF $\alpha$  antagonists, it has been shown to be effective, both as monotherapy and in combination with methotrexate, in reducing signs and symptoms of RA (Breedveld et al. 2006; Ebell and Kripke 2006; Weinblatt et al. 2006).

*Mechanism:* Adalimumab binds specifically to TNF $\alpha$  and blocks its interaction with the p55 and p75 cell surface TNF receptors, thereby interfering with endogenous TNF $\alpha$  activity (Bang and Keating 2004).



## **2.6 Rheumatoid Cachexia**

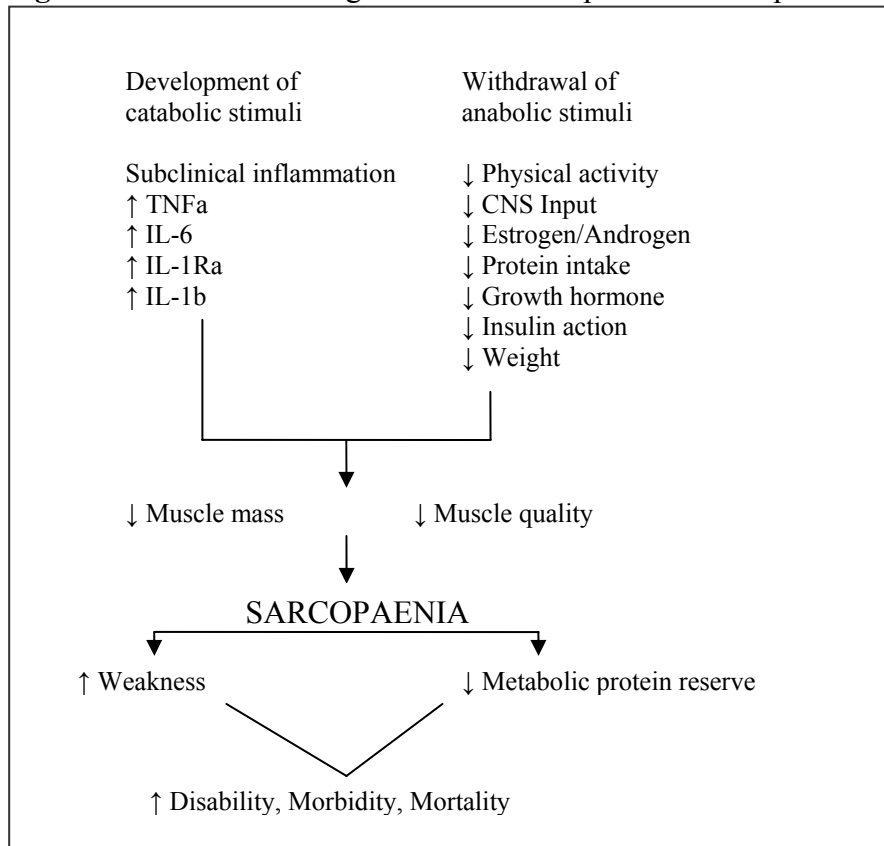
Skeletal muscle mass along with visceral, immune, brain, marrow and bone mass, constitute the body cell mass (BCM). This accounts for about 95% of the total metabolic activity of the human body (Cahill 1970). Loss of BCM leads to reduced energy expenditure, compromised muscular strength, reduced balance and movement ability, and impaired immune function (Walsmith and Roubenoff 2002). BCM losses greater than 40% of baseline values associate with almost certain death (Dewys et al. 1980; Kotler et al. 1989).

### **2.6.1 Sarcopaenia**

Loss of BCM is associated with qualitative and quantitative declines in skeletal muscle mass, a condition also occurring with normal ageing, termed sarcopaenia. This term has been introduced to denote the decreases in muscle mass and muscular strength occurring with normal ageing (Rosenberg 1989). Published data systematically have shown that both BCM and muscular strength are lower in older adults when compared to middle-age and young individuals (Gallagher et al. 1997a; Gallagher et al. 1997b), (Frontera et al. 1991; Kallman et al. 1990). The exact mechanisms of sarcopaenia are not yet fully understood; some of the potential contributing factors are presented in Figure 7. One of the biological mechanisms implicated in the development of sarcopaenia, namely the age-related decrease in growth hormone, is also the cause of another ageing process, known as somatopause (usually begins in mid-forties). Although sarcopaenia does not encompass BCM changes occurring as result of disease, the evidence suggests that it

increases morbidity and decreases quality of life and life expectancy (Argiles et al. 2006a).

**Figure 7.** Potential aetiologies for the development of sarcopenia.

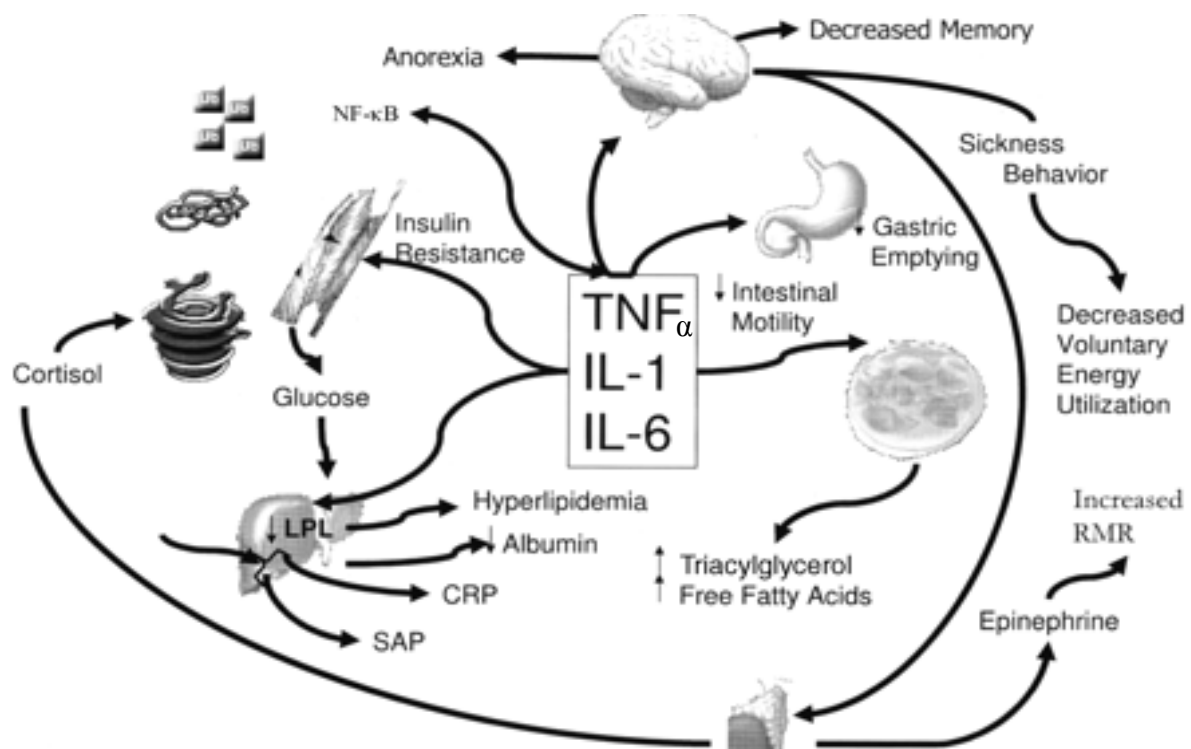


### 2.6.2 Cachexia

Cachexia is the rapid loss of muscle mass (Morley et al. 2006) and is exemplified by an accelerated involuntary BCM loss, predominantly in the skeletal muscle, occurring as a result of illness (Grunfeld and Feingold 1992) such as cancer (Muscaritoli et al. 2006; Stewart et al. 2006) or HIV (Chang et al. 1998; Scevola et al. 2000). Cachexia is inversely correlated with survival time (Argiles et al. 2006a) and eventually leads to increased mortality (Morley et al. 2006). One of the major factors leading to cachexia is

the overproduction of pro-inflammatory cytokines (Chang et al. 1998; Metsios et al. 2006; Morley et al. 2006; Rall and Roubenoff 2004). Figure 8 depicts the central metabolic effects of the pro-inflammatory cytokines of interest, one of which is the increased REE which leads to the development of cachexia.

**Figure 8.** The metabolic effects of pro-inflammatory cytokines.



Adopted from: <http://www.ajcn.org/cgi/content/full/83/4/735/F1>

LPL=lipoprotein lipase; SAP, serum amyloid protein; CRP, C-reactive protein.

### 2.6.3 Resting Energy Expenditure and Chronic Inflammation

The balance between the rate of degradation and re-synthesis of intracellular proteins in each cell is very important, because if this balance is not sustained, it can result in significant loss in BCM (Lecker et al. 1999). Chronic inflammatory diseases alter the balance between protein degradation and re-synthesis, resulting in an elevation in REE as

well as a net efflux of amino acids from muscle to liver, and a shift of liver protein synthesis away from albumin but towards production of acute phase proteins. This shift, in long term, is deleterious because it inhibits anabolism and can cause cachexia (Walsmith and Roubenoff 2002).

This powerful influence on protein (enhanced protein catabolism) from chronic inflammation, exerts a significant alteration on energy metabolism and causes hypermetabolism (i.e., increased REE) and relative anorexia; these conditions affect whole body composition and match those of other chronic illnesses as well as normal ageing (Roubenoff and Kehayias 1991). The hypermetabolism is caused by the constant protein degradation and is attributed to excess pro-inflammatory cytokine production, from peripheral blood mononuclear cells. Production of  $\text{TNF}\alpha$  and  $\text{IL-1}\beta$  during the initiation of the acute phase response in inflammatory diseases, such as RA, causes several systemic changes which, in turn, contribute to the above mentioned metabolic alterations (Dinarelli 1984).

#### 2.6.4 Rheumatoid Cachexia

The term rheumatoid cachexia is used specifically for patients with RA and dates back to the original cases of RA (Paget 1873). In RA, BCM loss has been associated with muscle weakness and limited mobility, thus reduced functional ability and quality of life (Munro and Capell 1997; Westhovens et al. 1997). However, rheumatoid cachexia is also accompanied by little or no weight loss in the presence of stable or increased fat mass: this has led to the introduction of the term “rheumatoid cachectic obesity” (Rall and Roubenoff 2004) and may be of great importance. It essentially suggests that RA patients

with normal or near normal body mass index (BMI) may have a significantly higher fat component in their body composition than age, sex and BMI-matched controls. Relevant research from our laboratory has found that this is indeed the case (Metsios et al. 2005; Stavropoulos-Kalinoglou et al. 2007). This increased fat mass may be responsible for intensification and perpetuation of the inflammatory response, as adipocytes (as well as the liver) represent one of the predominant sources of IL-6 in the body. It may also relate to the well-described association of RA with increased overall and cardiovascular mortality (Kitas and Erb 2003).

#### 2.6.5 Rheumatoid Cachexia and Energy Expenditure

REE accounts for ~70% of daily energy expenditure (Poehlman 1989), and depends on nutritional habits (Walsmith and Roubenoff 2002), gender (Gallagher et al. 1996), age (Ryan et al. 1996), FFM (Seals et al. 2001), and the individuals' levels of aerobic fitness (Sjodin et al., 1996). REE levels have also been found to be significantly depended on BCM (Poehlman et al. 1995a).

The human body is comprised of fat-free and fat mass. FFM is divided into BCM, and extracellular fluid (such as glucose, amino acids and ions) and solids and thus, we can calculate BCM by subtraction:

$$\text{BCM} = \text{fat-free mass} - (\text{extracellular fluid} + \text{extracellular solids})$$

Furthermore, the major parts of BCM are skeletal mass, visceral, and immune system FFM. Skeletal muscle cell mass is directly associated with strength and functional status

(Frontera et al. 1991) while both skeletal muscle mass and visceral cell mass determine the energy demands of the human body (Moore 1980). In addition, BCM accounts for 95% of body metabolic activity (Cahill 1972); loss of BCM is directly associated with reduced strength and functional status as well as decreased energy demands (Metsios et al. 2006; Rall and Roubenoff 2004). In all clinical situations that BCM has been studied, a loss of greater than 40% from baseline leads to death; loss of less than 40% of baseline, although not fatal, compromises functional ability and immune function (Walsmith and Roubenoff 2002).

During prolonged, uncontrolled, active inflammatory stages of the disease, patients with RA tend to lose weight and become cachectic. Roubenoff et al. (1990) found that individuals with RA experience negative nitrogen balance which is a mechanism underlying muscle protein degradation and thus, muscle wasting (cachexia). This was further reinforced by the discovery that  $\text{TNF}\alpha$  and  $\text{IL-1}\beta$  modulate lean body mass in RA, enhancing protein turnover towards catabolism (Roubenoff et al. 1994; Roubenoff et al. 1992). Moreover, data from the same research group showed that the enhanced protein degradation caused a significant increase in REE, evaluated via indirect calorimetry, which was directly related to the overproduction of these cytokines. Research attempts were also made in order to find other mechanisms causing hypermetabolism in RA, particularly growth hormone secretion; it was found that the kinetics of growth hormone do not account for rheumatoid hypermetabolism. However, a significant finding was that an increase in protein breakdown and REE is evident even when patients have a well-controlled disease status (Walsmith et al. 2004).

## 2.7 Specific Mechanisms of Rheumatoid Cachexia

The exact pathophysiological mechanisms underlying the development of rheumatoid cachexia are not yet fully understood. Two of the most widely discussed mechanisms, however, are excessive pro-inflammatory cytokine production and physical inactivity.

### 2.7.1 Cytokines

TNF $\alpha$  was first identified as ‘cachectin’ after its catabolic function. TNF $\alpha$  is involved in biological processes, which go well beyond its pro-inflammatory functions (Vassalli 1992), possibly due to the differential bioactivities of its soluble and transmembrane form (Grell 1995). TNF $\alpha$  is intimately involved in the metabolic abnormalities observed in RA, leading to significant skeletal muscle wastage and augmentation in energy expenditure and, thus, the occurrence of rheumatoid cachexia. In a synergistic manner TNF $\alpha$  and IL-1 $\beta$  significantly affect muscle metabolism by enhancing protein catabolic processes (Lecker et al. 1999). Since the balance of protein degradation and re-synthesis is not maintained within the muscle, continuing cytokine overdrive tips the balance towards protein breakdown (Rall and Roubenoff 2004). This is in line with more recent data which revealed that TNF $\alpha$  and IL-1 contribute to central nervous system side effects (such as pain), increase muscle metabolism and bone marrow suppression, all of which are present in chronic inflammatory diseases (Arend 2001).

The exact biochemical mechanisms by which skeletal muscles undergo rapid protein loss in response to cytokine overproduction, have been frequently studied *in vivo* and *in vitro* since muscle wasting characterises many chronic serious diseases (e.g.,

cancer, renal failure, HIV and RA). Enhanced muscle proteinolysis seems to occur through the ubiquitin-proteasome pathway (Mitch and Goldberg 1996), as protein catabolism is targeted by conjugation to ubiquitin (Lecker et al. 1999). TNF $\alpha$  is thought to stimulate muscle catabolism via an NF-kappaB-dependent process that increases ubiquitin conjugation to muscle proteins (Li et al. 2003). Through a cascade of processes, TNF $\alpha$ , bound to surface receptors, activates the transcriptional NF-kB pathway leading to the degradation of protein (Guttridge et al. 2000; Langen et al. 2001). This pathway is important in chronic inflammatory diseases as it is engaged in mediating the production of IL-1 and TNF $\alpha$  as well as their effects on target cells after they have bound to cell surface receptors (Arend 2001).

TNF $\alpha$  may also cause reduced protein synthesis through reduced insulin action in RA, as it interferes with the insulin receptor signalling pathway (Hotamisligil et al. 1994). Several studies suggest that RA associates with reduced peripheral insulin action (Paolisso et al. 1991; Svenson et al. 1987). Insulin triggers intracellular enzyme activity that facilitates protein synthesis by increased amino acid transport through the plasma membrane, RNA cellular levels, and protein formation by ribosomes (McArdle et al. 2001).

IL-1 and IL-6 have also been proposed as mediators of muscle protein degradation in different catabolic conditions. However, the proteinolytic mechanisms involved are not yet fully understood (Garcia-Martinez et al. 1995; Llovera et al. 1995).

Human studies demonstrate the association between excess TNF $\alpha$  and IL-1 $\beta$  with rheumatoid cachexia. By blocking either TNF $\alpha$  with a recombinant soluble TNF $\alpha$  receptor antagonist or IL-1 $\beta$  with a recombinant IL-1 receptor antagonist (IL-1Ra), it was found that blocking TNF $\alpha$  alone only moderately reduced the loss of skeletal muscle



weight. Blocking both TNF $\alpha$  and IL-1 $\beta$  was more effective in preventing undue muscle wasting, indicating that TNF $\alpha$  is an important element in this process, but not on its own (Roubenoff et al. 2001). It remains unclear whether these cytokines endorse protein degradation or restrain protein synthesis, and whether their effects are direct or through a network of many other hormones and cytokines.

### *Smoking*

Although smoking alone is not a factor that may cause cachexia, it has been found that smokers experience enhanced REE compared to non-smokers (Collins et al. 1994). As a result they lose weight easier than non-smokers; however, it was suggested that this was mainly loss in FFM and not fat mass (Akbarbartoort et al. 2005). If this is the case, RA patients who are smokers will experience even more increased REE from the synergistic effect of smoking and rheumatoid-related hypermetabolism. This is a hypothesis worth investigating in order to prevent unwanted health consequences.

### 2.7.2 Physical Inactivity

RA is a chronic progressive disease of the joints associated with systemic involvement and deformity. Structural damage in the musculoskeletal system can be developed within the first two years of the disease (Kavuncu and Evcik 2004). Pain, joint stiffness and inflammation, fatigue and reduced muscle strength are all elements of RA symptomatology (Hall et al. 2004). Physical activity can, therefore, be significantly compromised in RA patients.

In general, reduced physical activity leads to muscle weakness, inability to exercise, insulin resistance and energy balance variations. Bed-rest studies reveal that

lack of effective muscle stimuli decreases the turnover rates of muscle and whole-body proteins, with a prevailing inhibition of protein synthesis (Biolo et al. 2005). Patients' fear of aggravating the disease and the traditional approach of rheumatology health professionals to recommend exercise restriction, owing to concerns about enhancing the joint inflammation and joint damage (Anandarajah and Schwarz 2004) may also account for the inactive lifestyle of these individuals (Scott and Wolman 1992). These factors might also be contributing to the initiation and/or progression of rheumatoid cachexia.

## **2.8 Reversing Rheumatoid Cachexia**

### **2.8.1 Anti-Tumor Necrosis Factor Treatment**

Research investigating anti-TNF $\alpha$  medication has focused on its effects in improving the status of various diseases, such as psoriatic arthritis (Soriano and McHugh 2006; Tobin and Kirby 2005) and ankylosing spondylitis (De Keyser et al. 2004; De Keyser et al. 2006) and RA (Bongartz et al. 2006; Navarro-Sarabia et al. 2005). As indicated, rheumatoid cachexia is a cytokine-driven hypermetabolic abnormality (Roubenoff et al. 1994) which enhances protein catabolic process and REE leading to muscle wasting. It has been postulated that the newly developed anti-TNF $\alpha$  medication may reverse this phenomenon (Metsios et al. 2006; Rall and Roubenoff 2004). Only one study that has been recently published, concluded that anti-TNF $\alpha$  treatment is not superior to methotrexate treatment in reversing cachexia in early RA patients (Marcora et al. 2006). In a very small number of participants (n=6) who gained weight (44% of the increased weight was FFM), it was suggested that anti-TNF $\alpha$  might have a positive effect in

reversing this metabolic abnormality. However, the very small number of participants does not allow for definite conclusions. In addition, this study investigated the effects of the treatment on body composition measurements alone, without simultaneously taking into account and/or assessing for factors that significantly affect body composition (i.e. REE and physical activity). Also, no experimental studies to date, have investigated the effects of this treatment on longstanding patients with RA.

### 2.8.2 Exercise and Lifestyle Changes

Muscle growth and/or maintenance require sufficient physiological stimuli. The beneficial effects of strength exercise in the maintenance of adequate age-related strength levels have been well-established. Strength training programmes elicit significant improvements in muscle strength levels in healthy adults (Broeder et al. 1992) of all ages (Boshuizen et al. 2005). Also, significant improvements in muscular strength may be attained with either low-volume or high-volume programmes (Marx et al. 2001).

Resistance training causes hypertrophy in muscle fibres of all types, particularly those designated as fast-twitch or type II fibres; the area occupied by these fibres may increase by as much as 90%, mainly due to increased rates of protein synthesis and the associated augmentation in myofibril size (Koutedakis et al. 2006). Specifically for RA, strength training has been identified among the most important non-pharmacological treatments. Conventional resistance exercise programmes - consisting of low impact isometric and a range of motion exercises - have been repeatedly utilized in RA patients (Munneke et al. 2004). The application of such low impact regimens was mainly due to the presumed harmful effects of more intensive exercise programs on disease activity and

joint structures (Scott and Wolman 1992). However, RA studies have clearly suggested that high intensity exercise programmes are more efficacious in increasing muscular strength, compared to any low intensity training regime and range of motion exercises, without aggravating the disease symptoms (Bostrom et al. 1998; de Jong et al. 2003; Ekdahl et al. 1990; Stenstrom et al. 1996). The use of high intensity exercise might even decelerate joint damage in individuals with RA compared to non-exercising RA patients (de Jong et al. 2004; Nordemar et al. 1981). Nevertheless, it is important to note that patients with extensive structural damage should refrain from activities that include loading of the damaged joints (Munneke et al. 2005). Interestingly, despite the effectiveness and safety of moderate and high intensity exercise, RA patients, physiotherapists and rheumatologists expect more positive results from low intensity exercise programmes (Munneke et al. 2004). Regarding rheumatoid cachexia, it has been recently shown that well-constructed resistance training programmes are effective and safe interventions for stimulating muscle growth in patients with RA and may even reverse rheumatoid cachexia (Mancora et al. 2005).

Apart from strength training, adopting a more active lifestyle can result in health benefits in conditions such as diabetes (Laaksonen et al. 2005) and the metabolic syndrome (Daskalopoulou et al. 2004); probably this would also be the case in RA. Physically active individuals can maintain a higher level of physical functioning, reduce the rate of age-related muscle wasting (Fiatarone et al. 1994), and improve life-satisfaction and well-being (Ohta et al. 2004). The effects of lifestyle changes in relation to rheumatoid cachexia are also a matter of future investigation.

### 2.8.3 Dietary Interventions

Research on diet and RA has mainly focused on the allergic effects that food has on RA (Buchanan et al. 1991; Lunardi et al. 1988) or the impact of specific nutrients in aggravating or ameliorating the disease characteristics (Bourne et al. 1985; Stamp et al. 2005), (Beri et al. 1988; Kjeldsen-Kragh 1999).

Only one study (Marcora et al. 2005) has directly investigated a specific diet to prevent rheumatoid cachexia. The study provided as intervention a mixture of beta-hydroxy-beta-methylbutyrate, glutamine and arginine as treatment. Nonetheless, although the studied diet was better tolerated by the participants compared to a placebo diet, it did not reverse cachexia.

It can be argued here, that effective diet alone, is not an effective intervention to reverse rheumatoid cachexia. The reason is that if the protein intake is not needed by the body – e.g., for the development of muscle mass – it is removed. In contrast, daily protein intake of about 1.5 g per kg of body mass is necessary during the period of strength training (Lemon 1995). This is supported by studies on other groups of individuals; very old men and women have benefits from the use of a protein-calorie supplement along with strength training which evidently is associated with greater strength and muscle mass gains than did the use of placebo (Evans 2004). However, every diet has to meet individual needs according to the required amount of protein intake (Lemon 1996), given that animal protein consumption is related to increased risk for cardiovascular disease, due to the increased levels of saturated fat (Hu et al. 1999).

### **3. CHAPTER 3: RATIONALE AND AIMS OF THE PRESENT STUDY**

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RA is accompanied by a cytokine-driven hypermetabolic state which significantly compromises health and life quality but also leads to increased mortality. The hypermetabolism in relation to RA, however, has not been extensively investigated leaving many significant research questions unanswered:

1. Given the hypermetabolic state of RA and the significance of REE as an assessment, are existing prediction equations accurate in RA patients?
2. If not, what would be the components and accuracy of predictive equations developed specifically for RA?
3. Does smoking further enhance hypermetabolism in RA patients?
4. Since hypermetabolism in RA is at least in part due to excessive TNF $\alpha$ , is anti-TNF $\alpha$  biologic therapy effective in preventing or reversing rheumatoid cachexia?

The aims of the present study, therefore, were:

1. To evaluate widely-used REE predictive equations against actual measured REE in RA patients
2. To develop new, RA specific prediction equations for RA patients
3. To investigate if smokers with RA have higher REE than non-smokers with this disease
4. To investigate if anti-TNF $\alpha$  treatment prevents or reverses rheumatoid cachexia

The specific hypotheses addressed in the studies presented in this Thesis were that:

1. The existing REE prediction equations are not accurate in the RA population

2. Disease activity / systemic inflammation is likely to be a significant component of any RA-specific REE prediction equation
3. REE is significantly higher in RA smokers compared to non-smokers
4. Anti-TNF $\alpha$  treatment prevents or reserves rheumatoid cachexia.

## 4. CHAPTER 4: METHODS

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### 4.1 Participants

To test our hypotheses, we recruited in total 68 RA patients. In our first study we used data from 60 patients, in the second study we used 53 patients (after excluding the ex-smokers) while in the third, we used 20 patients, in whom anti-TNF $\alpha$  was prescribed. All patients met retrospective application of the revised 1987 ACR classification criteria (Arnett et al. 1988) and were recruited consecutively from rheumatology outpatient clinics of the Dudley Group of Hospitals, NHS Trust, in the UK. Volunteers formed two separate cohorts as follows:

#### Cohort 1

This cohort consisted of the total 68 consenting volunteers (female=48). Their data were used in the first (n=60) and second (n=53) studies and therefore, demographic and anthropometric data are presented in two different chapters: Chapter 1 (Tables 3 and 4), Chapter 2 (Table 7). In addition, 25 apparently healthy volunteers were recruited from hospital and laboratory personnel to form the control group. The latter group was divided into two sub-groups, each serving as control group in two different projects (15 and 10 individuals in Chapters 1 and 2, respectively). The demographic and anthropometric characteristics of these participants appear in the relevant chapters (Chapter 1: Table 3 and Chapter 2: Table 7).

RA participants had to be on stable medication for at least three months prior to assessment. Exclusion criteria included: previous/current thyroid disorders,



malabsorption, pregnancy, diarrhoea, proteinuria, abnormal liver function tests, obstructive or restrictive lung disease, congestive heart failure or current infection. Information was given to all participants in written format (Appendix 1), and a special follow-up visit was arranged in the rheumatology research unit for final consent and assessment. The study had prior approval by the ethics committees of Dudley Group of Hospitals and of the University of Wolverhampton.

All participants attended a single two-hour laboratory session after visiting the data collection site early in the morning (8:00-9:00 a.m.) following a 12-hour overnight fast. Demographic and anthropometrical characteristics were recorded first, REE was measured next and a blood sample with assessment of the disease activity [disease activity score 28 (DAS28) and functional capacity [health assessment questionnaire (HAQ)] were performed last. DAS28 and HAQ were always obtained by the same specialized nurse.

## Cohort 2

Twenty patients of the total 68 patients were used in the third study. These patients were recruited because they had a clinical indication for treatment with a biological anti-TNF $\alpha$  therapy as per current BSR/NICE guidelines (Deighton 2005). These patients formed the second cohort of RA participants however, their data were also used in Cohort one. Patients were recruited consecutively from specialist nurse-led clinics at the Department of Rheumatology, Dudley Group of Hospitals NHS Trust, UK, where all patients to be commenced on these drugs, are screened and educated. Written informed consent (Appendix 2) was obtained from all participants after full explanation of the procedures

involved. The demographic and baseline anthropometrical characteristics of the 20 participants are shown in Chapter 3, Table 8.

Each participant attended, fasting, between 8:00 and 9:00 am, and underwent an identical assessment on three separate occasions. The baseline assessment (Time-1) occurred within two weeks prior to the initial anti-TNF $\alpha$  treatment. The second (Time-2) and third (Time-3) assessments were performed two and 12-weeks after the drug had been introduced. At all visits, anthropometrical characteristics were recorded prior to the REE measurement followed by a fasting blood sample. The DAS28 and HAQ completed the assessment; these were obtained, at all times, by the same specialised nurse.

## **4.2 Materials and Methods**

### **4.2.1 Actual Resting Energy Expenditure**

Actual REE was measured via indirect calorimetry, adhering to well-described methodological standards (Compher et al. 2006). Patients were instructed the day before the assessment to avoid excessive consumption of food as well as to be 12-hours fasted. Participants rested for a 20-min period prior to the measurement in a semi-darkened, quiet and thermoregulated (22 °C) room. An automated gas analyser (Metalyzer, Cortex Biophysik, Borsdorf, Germany), calibrated before each test using standard gases of known concentration, was used to record respiratory parameters every 20 sec, while subjects inspired room air through a free-breathing face mask. Data were collected over a period of 40 min with the participants being instructed to refrain from sleeping or hyperventilating. Mean values of REE for that period, excluding the first and last five minutes, were calculated using the Weir equation (Weir 1990).

#### 4.2.2 Predicted Resting Energy Expenditure

Commonly used equations (Chapter 1, Table 5) in clinical practice were also utilized to predict REE. Most of the equations incorporated in the prediction easily measurable values (age, weight, height) while others were based on FFM.

#### 4.2.3 Body Composition

Standing height was measured to the nearest 0.5cm (Seca Stadiometer 208). Body mass was measured to the nearest 0.1 kg and body composition was evaluated using a segmental body composition analyzer (Tanita BC418-MA). For the latter assessment, patients were asked to stand on the apparatus barefoot while holding the provided grips. The whole assessment lasted approximately 30 seconds. To obtain the body composition information, a small electrical current flows through the four electrodes (two on the feet and two on the hands). The current passes more rapidly through the FFM because, compared to fat tissue, it has a greater electrolyte content and hence, lower resistance. This can, then, be used to assess FFM and body fat percentage (Wells and Fewtrell 2006).

Bioelectrical impedance assessment is largely affected by the hydration status of the participant (Deurenberg 1996). This information was provided to all participants; we also reminded participants the day before the assessment not to drink large amounts of water prior to visiting the laboratory. Moreover, before all body composition assessments we have asked participants to report the amount of water they drunk at the morning of the assessment. All patients reported one or a maximum of two glasses of water before assessment.

#### 4.2.4 Contemporary Serological Disease Activity

##### 4.2.4.1 C Reactive Protein

The assessment of CRP as a serological marker of disease activity is very well established (Levinson 2006). The measurement of CRP uses the Vitros® 5.1 FS chemistry system and the multilayered slides; however, measurement is based on an enzymatic heterogeneous, sandwich immunoassay format. In this format, a derivative of phosphorylcholine is covalently bound to polystyrene polymer beads and the presence of calcium serves as a capture agent. Monoclonal anti-CRP antibody labelled with horseradish peroxidase serves as a signal generator. A drop of patient's sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. CRP in the sample binds to the phosphorylcholine-linked capture beads and the anti-CRP antibody labelled with horseradish peroxidase to form an insoluble sandwich complex in the first incubation. This is subsequently washed with a specific solution which also provides the hydrogen peroxide for the enzyme mediated oxidation of leuco dye. The reflection density of the dye is measured after a second wash giving a reflection density directly proportional to the concentration of CRP in the sample.

##### 4.2.4.2 Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) determination is based on a simple and inexpensive laboratory test that is frequently ordered in clinical medicine (Brigden 1998; Saadeh 1998). ESR is also a valid marker of inflammatory load in RA (Paulus and Brahn 2004). The test measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity. The ESR was measured via the Starrsed compact from Mechatronics BV, Netherlands.

#### 4.2.5 Contemporary Clinical Disease Activity

##### Disease Activity Score 28

Disease activity was measured with the use of DAS28 (Prevoo et al. 1995)]. This is a composite assessment consisting of the patient's assessment of overall health during the last week on a visual analogue scale, a 28 joint count and the current ESR. To identify the swollen and tender joints, the nurse had to squeeze the joints of the arms, elbows, hand and knees (Appendix 3) of every participant.

#### 4.2.6 Functional Capacity

The anglicised version of the 40-item Stanford Health Assessment Questionnaire (HAQ), (Kirwan and Reeback 1986) was used to measure functional disability. In this assessment, participants rate their ability (over the past week) to carry out 20 activities within eight aspects of daily living (dressing/grooming, rising, eating, walking, hygiene, reach, grip and errands/tasks) on a four-point scale from 'without any difficulty' to 'unable to do' (Appendix 4). For each aspect, patients also responded whether they received assistance from people or use specific devices. The HAQ is internally consistent ( $\alpha \geq 0.89$ ) and has excellent pre- to post-physician visit temporal stability ( $r = 0.99$ ).

#### 4.2.7 Tumour Necrosis Factor alpha

Serum was also collected for measurement of TNF $\alpha$  levels. The collected serum was kept in collection devices that were durable, leak-proof and constructed of non-absorbing plastics. This was frozen immediately at -70 °C, until analysed by multi-analyte Biochip Array Technology (Evidence analyzer, Randox, USA) on a single occasion for all

specimens. This analyser is a fully automated biochip array system that can perform simultaneously the quantitative detection of multiple analytes from a single patient.

#### 4.2.8 International Physical Activity Questionnaire

The long version of the international physical activity questionnaire (IPAQ) was utilized to record the physical activity of the RA patients with the 12-week period. The questionnaire is divided in five parts asking about the physical activities (job-related, transportation, housework, leisure time, and time spend sitting) that the participants have undertaken over the last seven days. The same nurse was always helping the patients to fill in the questionnaire. The IPAQ has been extensively used for research purpose while its validity and reliability has been assessed in 12 countries, including the UK (Craig et al. 2003). The written details that were provided to the participants as well as the questionnaire itself, appear in Appendix 5.

### 4.3 Statistical Analyses

The statistical methods used in the present research project are mainly parametric and non-parametric t-tests since the individual studies are cross-sectional (Studies 1 and 2) and longitudinal (Study 3); as such the observations are based on simple comparisons between the groups or between the different times of assessment. Moreover, in the first study the 95% limits of agreement have been also utilized since, as an approach, are more valid in assessing agreement between two estimates compared to the use of correlation coefficients and t-tests. Moreover, the development of a new equation which aims to predict REE in the RA population has been developed using ANCOVA. ANCOVA is also used in the second study to investigate the relative contribution of both smoking and disease activity on the prediction of REE.

## Study 1: Prediction of resting metabolism in rheumatoid arthritis

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### 1. Introduction

The assessment of energy expenditure is an important issue in clinical research as imbalances between energy intake and expenditure may cause health complications (Muller et al. 2003). The main indicator of human metabolism is REE (Poehlman 1989). REE can be significantly altered by infection and chronic disease (Bosaeus et al. 2002; Rall and Roubenoff 2004; Schwenk et al. 1996), such as RA. This is the most common type of inflammatory arthritis in adults, with an estimated prevalence of ~1% in Europe and North America (Symmons 2002).

RA is accompanied by a metabolic abnormality, referred to as rheumatoid cachexia. This manifests with excessive production of pro-inflammatory cytokines, particularly TNF $\alpha$ . Overproduction of TNF $\alpha$  enhances protein catabolic processes (Metsios et al. 2006; Rall and Roubenoff 2004) leading to decreased FFM and increased REE (Roubenoff et al. 1994; Walsmith and Roubenoff 2002). This is also accompanied by a trend towards higher accumulation of fat mass, leading to detrimental health effects (Rall and Roubenoff 1996).

REE provides significant information about energy expenditure and, thus, protein metabolism. Indirect calorimetry, the most accurate method of measuring REE, is time consuming, involves expensive equipment, and requires trained personnel (McClave and Snider 1992). Hence, several prediction equations have been developed to estimate REE from easily accessible variables (e.g., age, gender, height, weight) or FFM, the body's metabolically active tissue (Wang et al. 2000). These equations have been mainly based on data deriving from general populations.

In general, existing REE equations are easy to use, inexpensive and universally available. However, they may be misleading in RA as they do not take into account the metabolic changes associated with the disease (Bauer et al. 2004; Dickerson et al. 2002; Zauner et al. 2006). It seems, therefore, reasonable to suggest that the existing REE equations would under predict the actual REE in RA patients. Hence, the development of an equation based on RA-related variables (e.g. acute inflammatory markers) and anthropometrical characteristics (e.g. weight, age, or FFM) may be necessary.

We hypothesised that currently available REE predictive equations are inaccurate in patients with RA. Therefore, the aims of the present study were to: a) to evaluate the accuracy of widely used REE predictive equations in RA patients against measured REE, and b) to develop a more accurate, RA-specific, equation.

## **2. Methods**

### **2.1 Participants**

Sixty consenting volunteers (female=40) with RA, all meeting retrospective application of the revised 1987 ACR classification criteria (Arnett et al. 1988) were recruited consecutively from rheumatology outpatient clinics of the Dudley Group of Hospitals NHS Trust in the UK. Fifteen apparently healthy volunteers (female=9) were also recruited from hospital and laboratory personnel. Demographic and anthropometrical data for both groups as well as differences between male and female RA patients appear in Tables 3 and 4, respectively. RA patients had to be on stable medication for at least three months prior to assessment. Exclusion criteria in both groups were: previous/current thyroid disorders, malabsorption, pregnancy, diarrhoea, proteinuria, abnormal liver function, obstructive or restrictive lung disease, congestive heart failure,



and current infection. Information was given to all participants in written format, and a special follow-up visit was arranged in the rheumatology research unit for final consent and assessment. The study had prior approval by the local research ethics committee and the Dudley Group of Hospitals Research and Development Directorate.

## 2.2 Procedures

All participants attended a single two-hour laboratory session after a 12-hour overnight fast. The sequence of the assessments as well as the materials and methods for this project is explicitly explained in Methods, Materials and Methods. In brief, demographic and anthropometrical characteristics (bioelectrical impedance) were recorded first, REE (indirect calorimetry) was measured next and a blood sample (for CRP and ESR) with assessment of DAS28 were performed last. A significant number of the existing REE formulae (Table 5) were also utilized to predict REE.

## 2.3 Data Analyses

Routine pre-analyses were conducted using the Kolmogorov-Smirnov normality tests to detect if variables were normally distributed. Comparisons and correlations between RA patients and controls for variables that were not normally distributed, were conducted using non-parametric tests. The Bland and Altman method (Bland and Altman 1986) was utilised to assess agreement between all prediction equations and actual REE measured via indirect calorimetry. This analysis allows the calculation of bias (mean of the individual differences between estimates), the 95% limits of agreement ( $\pm 2$  standard deviations from the mean bias) and the percent coefficients of variation (percentage of error prediction). Linear regression analysis was used to determine which of the

measured anthropometrical data and the disease activity assessments (CRP, ESR, DAS28) affected REE in the RA sample. ANCOVA was used to develop a RA-specific REE prediction equation. Statistical analysis was performed with SPSS software (version 11.0, SPSS Inc, Chicago, IL). Statistical significance for all analyses was set at  $p < 0.05$ .

### **3. Results**

#### **3.1 Measured REE in RA and controls**

Demographic and anthropometrical data for the entire cohort are shown in Tables 3 and 4. REE did not differ significantly between the two groups (RA:  $1638.3 \pm 359.5$  vs. Controls:  $1576.5 \pm 203.8$  kcal/day,  $p > 0.05$ ). This was also the case for weight and BMI ( $p > 0.05$ ) but not for height ( $p < 0.05$ ). However, compared to RA patients, FFM was significantly increased ( $p < 0.05$ ) and age was significantly less ( $p < 0.001$ ) in the control group (Table 3). After adjusting for these two parameters and for presence of disease, it was found that disease and FFM ( $p < 0.001$ ) significantly influenced REE and that, on average, REE was higher in the RA patients.

**Table 3.** Mean $\pm$ std for all studied variables in rheumatoid arthritis patients and controls (n=10).

	Rheumatoid Arthritis	Controls
Age (years)	62 $\pm$ 11	40 $\pm$ 12**
Height (cm)	164.1 $\pm$ 9.6	166.3 $\pm$ 9.8
Weight (kg)	69.5 $\pm$ 16.9	70.4 $\pm$ 11.3
Body Mass Index (kg/m <sup>2</sup> )	26.5 $\pm$ 5.4	24.4 $\pm$ 2.7
Fat Free Mass (kg)	46.2 $\pm$ 12.2	51.7 $\pm$ 10.5*
Body Fat (%)	34.0 $\pm$ 8.4	24.3 $\pm$ 8.5**
C-reactive Protein (mg/L)	20.9 $\pm$ 24.8	---
Erythrocyte Sedimentation Rate (mm1st hr)	26.8 $\pm$ 23.6	---
Disease Activity Score 28	4.4 $\pm$ 1.4	---
Sulphasalazine	29/60	---
Hydroxychloroquine	18/60	---
Methotrexate	45/60	---
Azathioprine	2/60	---
Cyclosporin	4/60	---
Leflunomide	6/60	---
Biological Treatment	20/60	---
Steroids	13/60	---

\* significant different at p<0.05; \*\* significant different at p<0.001

**Table 4.** Mean $\pm$ std for all studied variables in male and female rheumatoid arthritis patients.

	Males (n=20)	Females (n=40)
Age (years)	62 $\pm$ 8	62 $\pm$ 12
Height (cm)	172.4 $\pm$ 9.7	160.2 $\pm$ 6.6**
Weight (kg)	82.8 $\pm$ 17.8	67.4 $\pm$ 17.0*
Body Mass Index (kg/m <sup>2</sup> )	27.5 $\pm$ 4.1	26.0 $\pm$ 5.9
Fat Free Mass (kg)	57.6 $\pm$ 12.4	40.5 $\pm$ 7.2**
Body Fat (%)	29.7 $\pm$ 5.6	36.5 $\pm$ 8.8*
C-reactive Protein (mg/L)	22.3 $\pm$ 20.8	20.2 $\pm$ 26.9
Erythrocyte Sedimentation Rate (mm1st hr)	35.0 $\pm$ 28.2	22.8 $\pm$ 20.0
Disease Activity Score 28	4.8 $\pm$ 1.3	4.2 $\pm$ 1.4

\* significant different at p<0.05; \*\* significant different at p<0.001

### 3.2 Agreement between actual measured and predicted REE in RA and controls.

All prediction equations studied, significantly underestimated measured REE (all at  $p < 0.001$ ) in the RA population; this was not the case for the control group (Table 5). Moreover, the 95% limits of agreement and percent coefficient of variation revealed that the mean REE difference and the bias between measured and predicted (from all formulae) REE were much wider in the RA group compared to controls (Table 5).

**Table 5.** Mean±std differences, 95%limits of agreement and percent coefficient of variation between measured and predicted resting energy expenditure from existing formulae (indicated by first author or organisation name) in the current rheumatoid arthritis patients and controls (n=10).

	Rheumatoid Arthritis			Controls		
	Mean±std (kcal/day)	LIM <sub>AG</sub> (kcal/day)	CV%	Mean±std (kcal/day)	LIM <sub>AG</sub> (kcal/day)	CV%
Measured REE	1638.3±359.5	---	---	1576.5±203.8	---	---
Harris-Benedict (1919)	1414.5±277.2**	-230.8 (472.2)	15.9	1575.0±241.4	-1.46 (390.1)	12.6
FAO/WHO/UNU (1985)	1459.6±163.3**	-185.8 (523.9)	17.3	1496.6±139.7	-79.8 (342.6)	11.4
Owen et al. (1986 and 1987)	1428.1±252.2**	-217.3 (537.8)	17.9	1439.5±228.4*	-136.9 (349.7)	11.8
Mifflin et al. (1990)	1330.5±289.9**	-314.9 (453.9)	15.6	1496.7±240.8	-79.8 (376.7)	12.5
ASPEN (2002)	1449.7±375.4**	-195.7 (526.0)	17.4	1455.8±266.1	-120.6 (475.8)	16.0
Cunningham (1991)	1500.2±264.1**	-146.3 (461.1)	15.0	1618.2±228.3	41.7 (368.7)	11.7
Predicted REE from BI	1344.4±340.2**	-309.2 (530.4)	18.2	1582.3±209.5	5.8 (285.6)	9.2
Luke and Schoeller (1992)	1509.6±244.5**	-136.9 (464.8)	15.1	1618.9±211.4	42.4 (352.1)	11.2
Ravussin et al. (1982)	1433.6±254.6**	-212.9 (462.5)	15.3	1560.8±220.7	-18.4 (362.4)	11.8
Ravussin et al. (1986)	1446.4±255.9**	-200.2 (462.3)	15.3	1560.6±221.2	-15.8 (361.5)	11.7
Elia (1992)	1425.9±258.1**	-220.6 (461.9)	15.4	1541.2±223.1	-35.2 (363.4)	11.9
McNeal et al. (1987)	1323.0±262.8**	-323.5 (461.3)	15.8	1440.4±227.2*	-136.0 (367.6)	12.4
Heymsfield et al. (1988)	1300.6±264.1**	-345.9 (461.1)	15.9	1418.6±228.3*	-157.9 (368.7)	12.5
Kasiwazachi et al. (1988)	1436.7±299.6**	-209.8 (462.6)	15.3	1570.5±258.9	-5.9 (404.1)	13.1

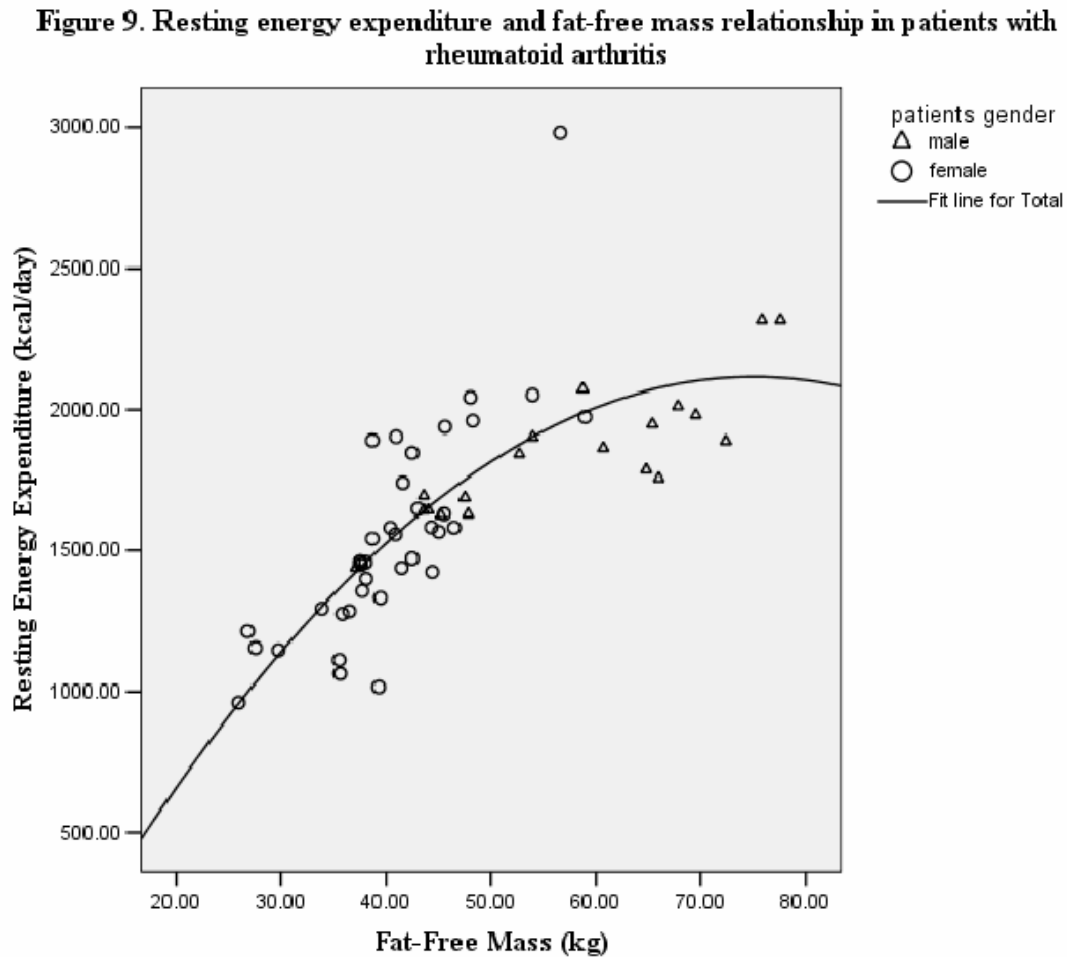
LIM<sub>AG</sub>=95% limits of agreement; CV%=percent coefficient of variation; BI=Bioelectrical Impedance.

\* significant different at p<0.05; \*\* significant different at p<0.001

### 3.3 Development of a new REE prediction equation in patients with RA.

In the total RA sample, REE correlated significantly with age ( $r=-0.33$ ,  $p<0.05$ ), height ( $r=0.62$ ,  $p<0.001$ ), BMI ( $r=0.57$ ,  $p<0.001$ ), FFM ( $r=0.76$ ,  $p<0.001$ , Figure 9) and weight ( $r=0.74$ ,  $p<0.001$ ). There were also significant correlations between REE and the contemporary serological estimations of RA disease activity CRP ( $r=0.56$ ,  $p<0.001$ ) and ESR ( $r=0.41$ ,  $p<0.001$ ). The latter parameters demonstrated a significant correlation between themselves ( $r=0.63$ ,  $p<0.001$ ). No significant correlations were observed between REE and any of the current medications ( $p>0.05$ ). Simple linear regression analysis revealed that FFM and weight had the highest prediction power in the total RA sample (both  $R^2=0.50$ ,  $p<0.001$ ), while gender did not significantly influence the prediction of REE ( $p>0.05$ ).

**Figure 9.** Resting energy expenditure and fat-free mass relationship in patients with rheumatoid arthritis.



The best prediction power was achieved after incorporating FFM ( $F_{1,54}=95.3$ ,  $p<0.001$ ) and CRP ( $F_{1,54}=38.9$ ,  $p<0.001$ ) which revealed a prediction power of  $R^2=0.76$ .

Hence, the following equation was developed:

$$\text{REE (kcal/day)} = 595 + (19.9 \times \text{FFM}) + (6.1 \times \text{CRP}).$$

where FFM=fat-free mass in kg, and CRP=c-reactive protein in mg/L.

Paired t-test revealed that this new equation had an almost identical mean with measured REE ( $1645.2 \pm 315.2$  and  $1645.5 \pm 363.1$  kcal/day,  $p > 0.05$ ) and a correlation coefficient  $r = 0.87$  ( $p < 0.001$ ). Moreover, 95% limits of agreement exposed no bias between predicted and measured REE ( $-1.3 \pm 350.8$  kcal/day), while the percent coefficient of variation was 10.8% (considerably lower than all other studied prediction equations).

#### **4. Discussion**

The findings of this study confirm our hypothesis that existing REE equations were inadequate in predicting REE in the current patients, possibly due to the RA-induced hypermetabolism, necessitating the development of a disease-specific equation. Hence, a new REE equation was developed with better prediction power and accuracy than any of the existing equations studied herein.

REE in our RA sample was only slightly higher than that of the control group and the difference was not statistically significant. Previous studies have clearly established that REE is increased in patients with RA compared to matched controls (Roubenoff et al. 1994; Roubenoff et al. 1992). The present study was not aimed at confirming this, so accurate matching of patients and controls was not necessary, which may be considered as a limitation of the present study. It is worth noting, however, that younger individuals should be able to exhibit higher REE compared to older individuals due to their FFM differences (Roberts and Dallal 2005). Despite the fact that the control group displayed significantly higher FFM levels, this was not accompanied by an equivalent significant increase in REE levels, implying that the present RA patients were in a hypermetabolic state. These phenomena are thought to be due to the excessive production of pro-



inflammatory cytokines, particularly TNF $\alpha$  (Roubenoff et al. 1994; Roubenoff et al. 1992).

Using the RA cohort, we evaluated the predictive accuracy of several existing REE formulae. Some of them incorporate only easily obtained anthropometrical information [(weight, height and age), (FAO/WHO/UNU 1985; Harris and Benedict 1919; Mifflin et al. 1990; Owen et al. 1987; Owen et al. 1986)], while others are based on FFM data (ASPEN 2002a; Cunningham 1991; Heymsfield et al. 1988; Kashiwazaki et al. 1988; Luke and Schoeller 1992; McNeill et al. 1987; Ravussin et al. 1982; Ravussin et al. 1986) which require the use of technologically advanced equipment. None of these existing and widely-used in clinical setting REE prediction equations were accurate in RA, although they were quite accurate in the controls. The most likely reason for this is that all formulae have been developed to predict resting metabolism in healthy individuals and that RA-related hypermetabolism is not taken into account. These findings re-enforced the need for the development of an RA-specific predictive equation. It is possible that similar disease-specific equations are required for other conditions with a significant, systemic, chronic inflammatory component, such as inflammatory bowel disease.

In the course of developing such an equation, we found that the best prediction power was achieved by incorporating in our model FFM and CRP. FFM reflects the only biologically active metabolic component of the human body (Wang et al. 2000) and therefore the major determinant of REE prediction (Muller et al. 2004). CRP – due to its relatively short half-life of approximately 19 hours (Kao et al. 2006) – is an accurate measurement of the current systemic inflammatory load. However, despite the strong association between REE and weight observed herein, we chose not to include weight in

our prediction model. This is because, at the same weight, RA patients have significantly less FFM and more fat compared to healthy individuals (Stavropoulos-Kalinoglou et al. 2007). Hence, inclusion of weight in an RA-specific REE equation, would not accurately reflect the exact amount of the metabolically active tissue.

Interestingly, in this sample of RA patients, gender did not have a significant impact on REE, so development of gender-specific equations, such as the Harris-Benedict, was not necessary for the current RA patients. The reasons for this are currently unclear. It is possible that the REE of a female RA patient might exceed that of a male RA patient with the same anthropometrical characteristics. Females have proportionally more body fat than males. Adipose tissue is one of the main sources of inflammatory cytokines such as interleukin-6 and TNF $\alpha$  (Fain et al. 2004; Krogh-Madsen et al. 2004), the main determinants of CRP (Lagrand et al. 1999). So, a potential relatively lower REE (due to lower FFM) in an RA female, would be counterbalanced by a higher inflammatory drive (due to more adipose tissue) which evidently increases REE (Roubenoff et al. 1994). This possibility appears to be supported by sub-analysis of our results (data not shown), but needs to be further investigated in future studies involving contemporary cytokine measurements.

A possible limitation of the present study is the sample size used to develop the prediction REE formulae. However, although some studies used data from a larger number of participants (Derumeaux-Burel et al. 2004; Mifflin et al. 1990; Muller et al. 2004), others introduced REE prediction equations using sample sizes smaller than ours (Buchowski et al. 2002; Kien and Ugrasbul 2004; Owen et al. 1986). Another limitation may be that we did not validate the new equation using a larger, different sample of patients with RA. Moreover, the use of a more accurate apparatus for the estimation of

FFM, such as dual energy x-ray absorptiometry, would add more credence to the present findings. Within these limitations it is concluded that existing prediction equations underestimate levels of REE in RA patients, most probably because the systemic inflammatory load associated with the disease is not taken into account. The RA-specific equation developed in the present study is accurate and displays much improved REE prediction power compared to all previous REE formulae.

## **Study 2: Smoking and metabolism in rheumatoid arthritis**

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This Chapter has been published in the *Annals of Rheumatic Diseases* 2007 (Epub ahead of Print, doi:10.1136/ard.2006.068403).

### **1. Introduction**

REE, the main indicator of human metabolism, is a vital physiological function providing for bioenergetics, body composition and by-product removal, and is largely affected by perturbations of the body's internal and/or external environment (Poehlman 1989). Inflammatory disease (Metsios et al. 2006; Rall and Roubenoff 2004), cancer (Staal-van den Brekel et al. 1995), human immunodeficiency virus infection (Batterham 2005), critical illness (Zauner et al. 2006), as well as active (Collins et al. 1996) and passive (Metsios et al. 2007) smoking, are all factors that significantly increase REE and disturb normal metabolic processes.

RA, the commonest chronic inflammatory joint disease, is accompanied by a set of metabolic abnormalities, collectively referred to as rheumatoid cachexia. This manifests as significant increases in REE due to the excessive production of the pro-inflammatory cytokine TNF $\alpha$ , a cytokine which enables the binding of ubiquitin on proteins (Lecker et al. 1999) thus, enhancing protein degradation. As a result RA patients experience involuntary and rapid wasting of muscle mass (Roubenoff et al. 1994) with subsequent abdominal fat deposition (Stavropoulos-Kalinoglou et al. 2007). This in turn, leads to increased cardiovascular risk (Rall and Roubenoff 2004), compromised muscle strength, balance immune function and functional ability; as such, rheumatoid cachexia is a significant overall contributor to co-morbidity and reduced life-expectancy in RA (Walsmith and Roubenoff 2002). Ideally, any factors associated with

increasing metabolism in RA should be identified and, if possible, eliminated early in the course of the disease.

In healthy subjects, cigarette smoking significantly augments resting metabolism (Walker and Kane 2002) as it enhances catecholamine release (Yamawaki et al. 1997) and increases activation of the pituitary-adrenal axis (Fisher et al. 1997). The impact of smoking on REE has not been assessed in patients with RA. However, smoking has been implicated in the aetiology (Criswell et al. 2006; Klareskog et al. 2007), severity (Papadopoulos et al. 2005) and cardiovascular co-morbidity (Gerli et al. 2005; Turesson et al. 2006) of RA. The aim of the present study was to investigate whether RA patients who were classified as smokers had higher REE levels than their non-smoking counterparts.

## **2. Methods**

### **2.1 Participants**

Fifty three consenting volunteers (36 females, 17 males; 20 current cigarette smokers, 33 non-smokers) with RA, all meeting retrospective application of the revised 1987 ACR classification criteria (Arnett et al. 1988) were recruited consecutively from rheumatology outpatient clinics of the Dudley Group of Hospitals NHS Trust in the UK. The group of RA smokers was further divided into three subgroups according to the number of cigarettes smoked per day (light smokers: <5, moderate smokers: 5-9, heavy smokers:  $\geq 10$ ). RA patients had to be on stable medication for at least three months prior to assessment. To investigate whether our RA patients were hypermetabolic, we also recruited 10 apparently healthy volunteers (6 females, 4 males; 4 smokers, 6 non-smokers) from hospital and laboratory personnel. Inclusion and exclusion criteria for the

present participants appear in Table 6 while their demographic and anthropometrical data are shown in Table 7.

**Table 6.** Exclusion and exclusion criteria for both the RA and control groups.

Inclusion Criteria	Exclusion Criteria
Euthyroid Normal absorption Various ages/ anthropometrical characteristics	Pregnancy Diarrhoea Proteinuria Obstructive or restrictive lung disease Congestive heart failure Current infection Ex-cigarette smokers, or current cigar, Pipe or other substance smokers

Information was given to all participants in verbal and written format, and a special follow-up visit was arranged in the rheumatology research unit for final consent and assessment. None of the patients who were informed about the aims and methods of study declined participation. The study had prior approval by the local research ethics committee and the Dudley Group of Hospitals Research and Development Directorate.

## 2.2 Procedures

Demographic and anthropometrical characteristics were recorded first, REE was measured next and a blood sample with the assessment of DAS28 and HAQ were performed last. Standing height was measured to the nearest 0.5cm (Seca Stadiometer 208). Contemporary RA disease activity was also measured serologically using the ESR and CRP.

## 2.3 Statistical Analyses

Routine pre-analysis screening was used to detect the normal distribution of the studied variables. Comparisons and correlations for not normally distributed variables (i.e., disease duration and CRP) were conducted using non-parametric tests. Correlation coefficients (Pearson's and Spearman's) were employed to assess whether parameters previously reported to influence REE, i.e. gender, age, height, weight, BMI, FFM and CRP (Mifflin et al. 1990; Poehlman 1989; Rall and Roubenoff 2004; Roubenoff et al. 1994) showed associations with REE in the present RA patients. ANCOVA was used to assess the influence of specific parameters on REE. T-tests were utilized to assess differences of all studied parameters between RA smokers and non-smokers. One-way ANOVA was adopted to examine whether mild, moderate and heavy RA smokers had different levels of REE. All statistical analyses were conducted using SPSS (version 13.0.1, Chicago, Illinois), while the statistical significance was set at  $p < 0.05$ .

## 3. Results

Antropometrical characteristics from all volunteers appear in Table 7. In the entire cohort of RA patients ( $n=53$ ), REE showed significant correlations with age ( $r = -0.36$ ,  $p = 0.008$ ), height ( $r = 0.59$ ,  $p = 0.000$ ), weight ( $r = 0.71$ ,  $p = 0.000$ ), BMI ( $r = 0.51$ ,  $p = 0.000$ ), and FFM ( $r = 0.72$ ,  $p = 0.000$ ). In addition, REE significantly correlated with CRP and DAS28 ( $r = 0.36$ ,  $p = 0.008$  and  $r = 0.27$ ,  $p = 0.47$ , respectively). However, ANCOVA revealed that smoking was a more powerful predictor of REE ( $F_{1,49} = 6.8$ ,  $p = 0.012$ ) than CRP and DAS28 independently (CRP:  $F_{1,49} = 5.7$ ,  $p = 0.021$  and DAS28:  $F_{1,49} = 2.4$ ,  $p > 0.05$ ).

There were no significant differences between smokers and non-smokers in anthropometrical characteristics, CRP or DAS28 score (Table 7). In contrast, significant

differences were observed between RA smokers and RA non-smokers in REE ( $1718.1 \pm 209.2$  vs.  $1513.9 \pm 263.3$  kcal/day;  $p=0.000$ ) and physical function assessed by the HAQ ( $1.66 \pm 0.8$  vs.  $1.04 \pm 0.8$ ;  $p=0.01$ ). ANCOVA revealed that this REE difference was significantly attributed to the association of smoking/gender ( $F_{1,38}=4.3$ ,  $p=0.045$ ), but to neither of these factors alone (smoking  $F_{1,38}=0.59$ ,  $p>0.05$ ; gender  $F_{1,38}=0.14$ ,  $p>0.05$ ).

**Table 7.** Means $\pm$ std for all studies variables in all participants: total sample of rheumatoid arthritis (smokers and non-smokers) patients and controls.

	Total RA Sample (n=53)	<i>P</i>	Controls (n=10)	RA Smokers (n=20)	<i>P</i>	RA non-smokers (n=33)
Gender	17	---	4	5	---	12
Males						
Females	36		6	15		21
Age (years)	61 $\pm$ 11	0.00	40 $\pm$ 12	58 $\pm$ 12	0.07	64 $\pm$ 11
Height (cm)	162.7 $\pm$ 9.2	0.27	166.3 $\pm$ 9.8	164.5 $\pm$ 9.8	0.26	161.6 $\pm$ 8.8
Weight (kg)	69.5 $\pm$ 16.9	0.87	70.5 $\pm$ 11.3	72.5 $\pm$ 19.7	0.32	67.7 $\pm$ 15.0
Body Mass Index (kg/m <sup>2</sup> )	25.9 $\pm$ 4.9	0.36	24.4 $\pm$ 2.7	26.2 $\pm$ 5.8	0.69	25.7 $\pm$ 4.4
Fat Free Mass (kg)	45.1 $\pm$ 9.8	0.36	45.2 $\pm$ 9.2	45.8 $\pm$ 10.8	0.46	44.6 $\pm$ 9.4
C-Reactive Protein (mg/L)	18.6 $\pm$ 19.0	---	---	21.8 $\pm$ 18.5	0.22	16.6 $\pm$ 19.3
Disease Activity Score 28	4.3 $\pm$ 1.2	---	---	4.6 $\pm$ 1.5	0.14	4.1 $\pm$ 1.1
Disease Duration (years)	12.0 $\pm$ 10.5	---	---	11.5 $\pm$ 9.2	0.94	12.3 $\pm$ 11.3
Basal Metabolic Rate (kcal/day)	1590.9 $\pm$ 262.0	0.18	1473.2 $\pm$ 194.9	1718.1 $\pm$ 109.2	0.000	1513.8 $\pm$ 263.3
Health Assessment Questionnaire	1.3 $\pm$ 0.9	---	---	1.7 $\pm$ 0.8	0.01	1.0 $\pm$ 0.8

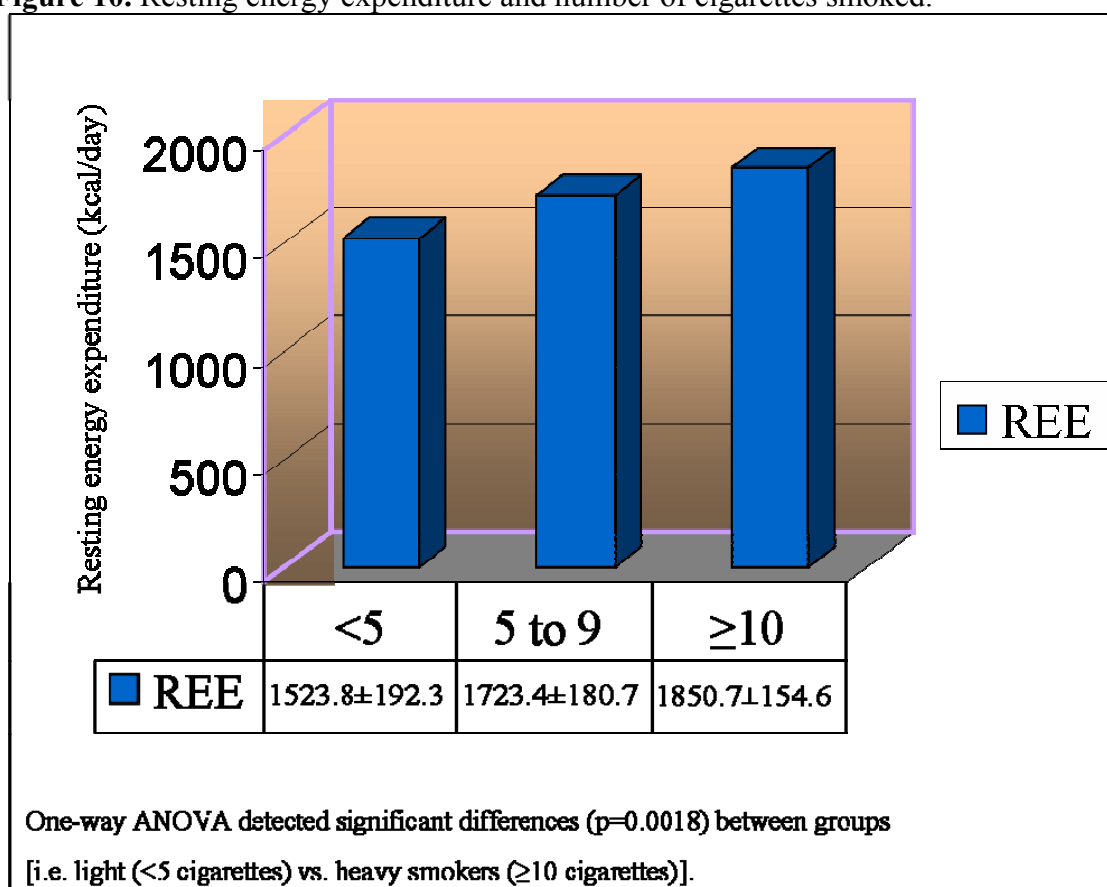
Within RA smokers, REE levels were significantly different between the light ( $1523.8 \pm 192.3$  kcal/day), moderate ( $1723.4 \pm 180.7$ ) and heavy ( $1850.7 \pm 154.6$  kcal/day) smokers ( $p=0.018$ ) (Figure 10); the significant difference occurred between light and



heavy smokers ( $p=0.016$ ). The number of cigarettes smoked correlated significantly with REE ( $r=0.61$ ,  $p=0.04$ ). A similar, but non-significant ( $p>0.05$ ), trend was found for the HAQ between light ( $1.4\pm0.9$ ) moderate ( $1.6\pm0.9$ ) and heavy ( $1.9\pm0.8$ ) smokers.

We have also compared mean $\pm$ std REE differences between RA patients and a small number of controls to assess whether the studied RA patients were characterised by hypermetabolism. After adjustment for gender, age, height, weight, BMI and FFM, ANCOVA showed REE to be higher in RA group compared to controls ( $1601.2\pm250.9$  vs.  $1444.1\pm234.1$  kcal/day, mean difference= $157.1$  kcal/day,  $p=0.000$ ). This difference was influenced by the disease ( $F_{1,49}=8.0$ ,  $p=0.007$ ) and FFM ( $F_{1,49}=5.0$ ,  $p=0.03$ ). Four RA participants were excluded from this ANCOVA due to missing FFM data.

**Figure 10.** Resting energy expenditure and number of cigarettes smoked.



#### **4. Discussion**

The aim of this study was to investigate whether RA smokers demonstrated different REE levels than those of their non-smoking counterparts. We found that REE in our entire RA sample was influenced by age, anthropometrical characteristics and FFM, as previously described in both normal (Mifflin et al. 1990; Poehlman 1989; Roubenoff et al. 1994) and diseased (Avesani et al. 2004; Lorefalt et al. 2004; Niskanen et al. 1993; Utaka et al. 2005) populations. These factors, together with disease activity (Rall and Roubenoff 2004) were important determinants of REE in the studied RA patients. This is in line with the current findings given the significant correlation between REE with CRP and DAS28. In our RA patients, however, smoking was a stronger REE predictor than CRP and DAS28.

The main finding was the significantly higher REE values observed in patients with RA who were current smokers, than those who did not smoke. This was despite the no significant differences in any of the important determinants of REE, even after adjustment for the variables that affect resting metabolism. Hence the observed REE differences can be attributed mainly, if not exclusively, to smoking.

The expected REE in healthy normal individuals with similar age and anthropometrical characteristics to those studied herein is about 1400 kcal/day (Cunningham 1991). We found that the present RA patients classified as non-smokers had a mean REE of 1513 kcal/day, i.e., about 8% higher than normal levels. We further found that RA patients who were smokers revealed a mean REE of 1718 kcal/day which is about 20% higher than normal. In RA patients, however, a REE increase of about 12% above normal levels has detrimental health implications, particularly when the extra energy derives from muscle catabolism (Metsios et al. 2006; Rall and Roubenoff 2004).

RA (Roubenoff et al. 1994) or smoking (Canoy et al. 2005) independently, can result in loss of fat-free mass which leads to compromised muscle strength and balance, functional mobility, and immune function (Walsmith and Roubenoff 2002). It seems reasonable to suggest, therefore, that the combination of RA and smoking would have damaging health consequences, which is partly supported by the present significant difference in HAQ between smokers and non-smokers.

The REE difference between RA smokers and non-smokers is supported by the present stepwise REE increases between light, moderate and heavy smokers, the direct significant correlation between the number of cigarettes smoked and REE, as well as the significant smoking/gender interaction in predicting REE. These elements suggest that smoking causes a dose-dependent increase in REE, which is in line with previous reports in normal populations (Collins et al. 1996; Walker and Kane 2002). Acute [e.g., immediate increase in oxygen demand (Heitzer and Meinertz 2005)] and chronic [e.g., elevated thyroxin levels (Fisher et al. 1997)] physiological changes due to smoking may account for increases in REE. The significant smoking/gender interaction in predicting REE also implies that if the sample is divided into male smokers, male non-smokers, female smokers, and female non-smokers, all four groups will reveal significant REE differences between them, a phenomenon also described in normal populations (Poehlman 1989; Walker and Kane 2002).

Results also revealed that RA patients had significantly higher REE values compared to controls. This may suggest that RA patients, were in a hypermetabolic state, as previously indicated for this population (Rall and Roubenoff 2004; Roubenoff et al. 1994). However, it is worth noting that controls were significantly younger. REE declines with age and, thus, younger individuals should have elevated REE (Roberts and

Dallal 2005). The small number of controls, however, is a limitation suggesting a cautious interpretation of the current REE differences.

Smoking in RA has attracted considerable research interest for many years. Smoking is a factor that has been unambiguously demonstrated in several studies to be associated with aetiopathogenesis of the disease (Hazes et al. 1990; Heliovaara et al. 1993; Hernandez Avila et al. 1990; Voigt et al. 1994), particularly in the context of concurrent genetic predisposition (Criswell et al. 2006; Klareskog et al. 2007). It appears to associate with worse disease activity and overall severity (Masdottir et al. 2000; Papadopoulos et al. 2005; Saag et al. 1997) as well as extra-articular manifestations including rheumatoid nodules (Nyhall-Wahlin et al. 2006), vasculitis (Turesson et al. 2006) and accelerated atherosclerosis (Gerli et al. 2005). Smoking has also been linked to imbalances in the production of TNF $\alpha$  and soluble TNF receptors, leading to a relative excess of TNF $\alpha$  (Glossop et al. 2006). This may be one of the mechanisms leading to the increased hypermetabolism specifically in RA smokers, as observed in the present study.

Although this study did not intend to identify the clinical implications of the increased REE due to smoking, it could be argued that the significant increase in HAQ, suggests that smoking negatively affects the patients' self-reported functional status. Smoking, together with age, gender, education, exercise, BMI and number of comorbidities, have previously been described as important determinants of HAQ disability (Sokka et al. 2003). Hypermetabolism in RA has also been linked to increased disability, reduced quality of life and premature mortality (Rall and Roubenoff 2004; Walsmith and Roubenoff 2002): it is likely that the additional smoking-induced elevation of REE will have further negative effects (e.g., increased protein catabolism) on the

health status of this population. The present findings add further reasons to suggest that the RA population has to be specifically targeted for smoking cessation (Gorman 2006).

### **Study 3: Anti-TNF $\alpha$ blockade and rheumatoid cachexia**

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#### **1. Introduction**

RA is a common chronic inflammatory condition, characterised by a symmetrical inflammatory polyarthritis and accompanied by systemic upset. It is associated with increased REE, loss of muscle mass and accumulation of body fat, a metabolic abnormality known as 'rheumatoid cachexia' (Paget 1873). This is observed even in well-controlled RA patients, who exhibit a 10% increase in REE above normal levels and a 13% loss of FFM compared with matched healthy individuals (Roubenoff et al. 1994; Roubenoff et al. 1992). This phenomenon has been attributed to increased pro-inflammatory cytokine production, in particular TNF $\alpha$  (Roubenoff et al. 1994).

Resistance training potentially stimulates muscular growth and prevents muscle wasting in RA (Marcora et al. 2005). It has also been suggested that anti-TNF $\alpha$  therapies may slow or even reverse rheumatoid cachexia (Metsios et al. 2006; Rall and Roubenoff 2004). A recent study (Marcora et al. 2006) demonstrated that body composition remained unchanged after anti-TNF $\alpha$  therapy, but did not assess important factors such as physical activity and protein intake, which significantly affect body composition. More importantly, it did not assess REE to investigate if anti-TNF $\alpha$  reversed RA-induced hypermetabolism, as previously suggested by reviews in the literature (Metsios et al. 2006; Rall and Roubenoff 2004). The aim of the present study was to investigate the early effects of anti-TNF $\alpha$  therapy on REE, body composition, physical activity and protein intake in RA patients.

## 2. Methods

### 2.1 Participants

The data of the patients from the second cohort were used for this project. These patients had a clinical indication for treatment with an anti-TNF $\alpha$  therapy as per current BSR/NICE guidelines (2001). Regarding the type of anti-TNF $\alpha$  medication, 10 have started Etanercept (25mg twice weekly), five patients started Infliximab (3 mg/kg during weeks 0, 2, 6, and subsequently, every 8 weeks) while the remaining five, Adalimumab (40 mg every second week). The study was approved by the local research ethics committee and the Dudley Group of Hospitals Research and Development Directorate. All patients met retrospective application of the revised 1987 ACR classification criteria for RA (Arnett et al. 1988). The demographic and baseline anthropometrical characteristics of the 20 participants are shown in Table 8.

**Table 8.** Anthropometrical characteristics of the rheumatoid arthritis patients.

	Mean $\pm$ std or Number (%)
Gender: Females	10 (50%)
Age (years)	61 $\pm$ 6
Height (cm)	166.9 $\pm$ 9.4
Time-1 Weight (kg)	79.4 $\pm$ 15.6
Rheumatoid Factor	18/20 (90%)
DMARDs	16/20 (80 %)
Methotrexate	14/20 (70 %)
Disease Duration (years)	17.3 $\pm$ 11.4
Note: DMARDs=disease modifying antirheumatic drugs	

## 2.2 Procedures

As mentioned above (Methods, Cohort 2), each participant attended fasting on three separate occasions and underwent identical procedures [baseline assessment (Time-1), second (Time-2) and third (Time-3) assessments were performed two and twelve weeks after anti-TNF $\alpha$  administration]. Anthropometrical characteristics (height and bioelectrical impedance) were recorded prior to the REE measurement (indirect calorimetry) followed by a fasting blood sample (CRP, ESR serum TNF $\alpha$ ); the assessment was completed with the assessments of clinical disease activity (DAS28), physical activity (IPAQ) and functional capacity (HAQ). Protein intake information was obtained through a simple food diary. The details of conducting all these assessments appear in Methods.

## 2.3 Statistical Analyses

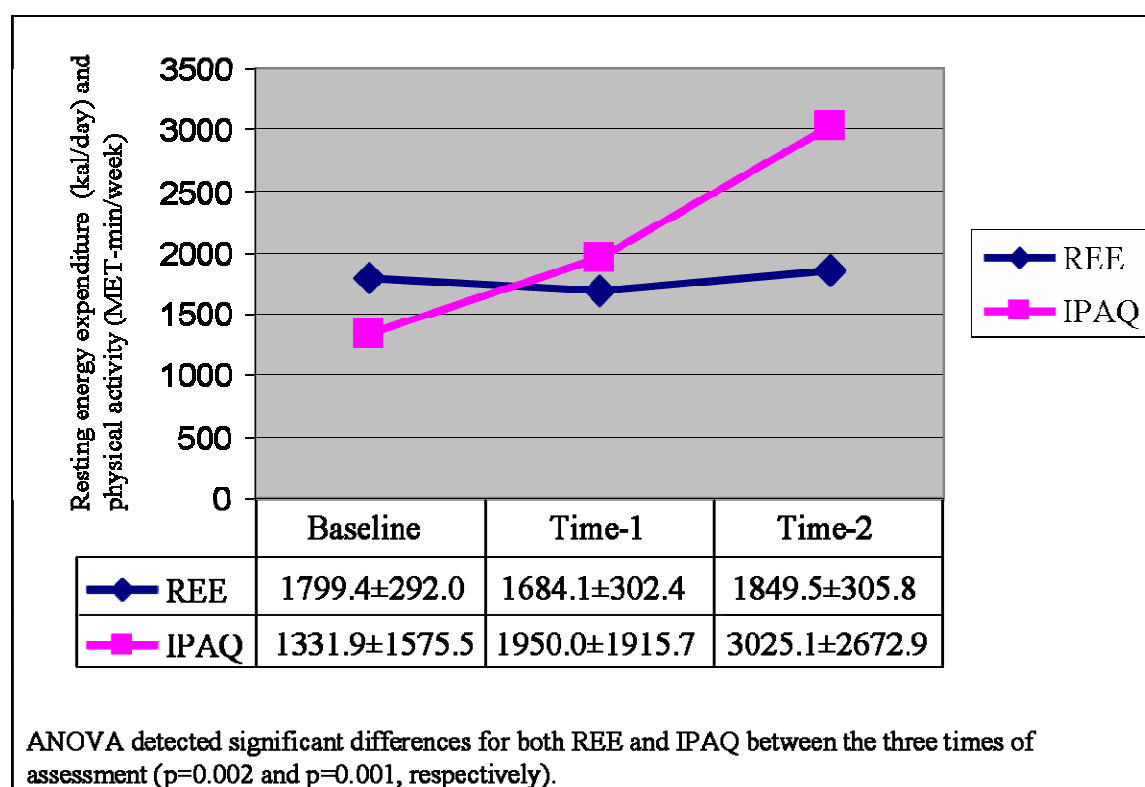
Values are reported as mean $\pm$ std. Preliminary analyses were conducted to detect if the studied variables were normally distributed. Repeated measures ANOVA were used to detect differences between different times of assessment (Time-1 vs. Time-2 vs. Time-3) for all studied variables. Physical activity and protein intake were also used as between-subject factors in order to detect the influence of each of them on the observed REE changes. Statistical significance was set at  $p < 0.05$ . All statistical analyses were performed with SPSS software (version 11.0, SPSS inc, Chicago, IL).



### 3. Results

For REE, repeated measures ANOVA revealed a significant difference between times of assessment (Time-1:  $1799.4 \pm 292.0$  vs. Time-2:  $1684.1 \pm 302.4$  vs. Time-3:  $1849.5 \pm 305.8$  kcal/day,  $p=0.002$ , Figure 11). This was also the case for IPAQ (Time-1:  $1331.9 \pm 1575.5$  vs. Time-2:  $1950 \pm 1915.7$  vs. Time-3:  $3025.1 \pm 2672.9$  MET-min/week,  $p=0.001$ , Figure 11). Results also revealed that IPAQ was a significant between-subject factor for the observed changes in REE ( $p=0.001$ ).

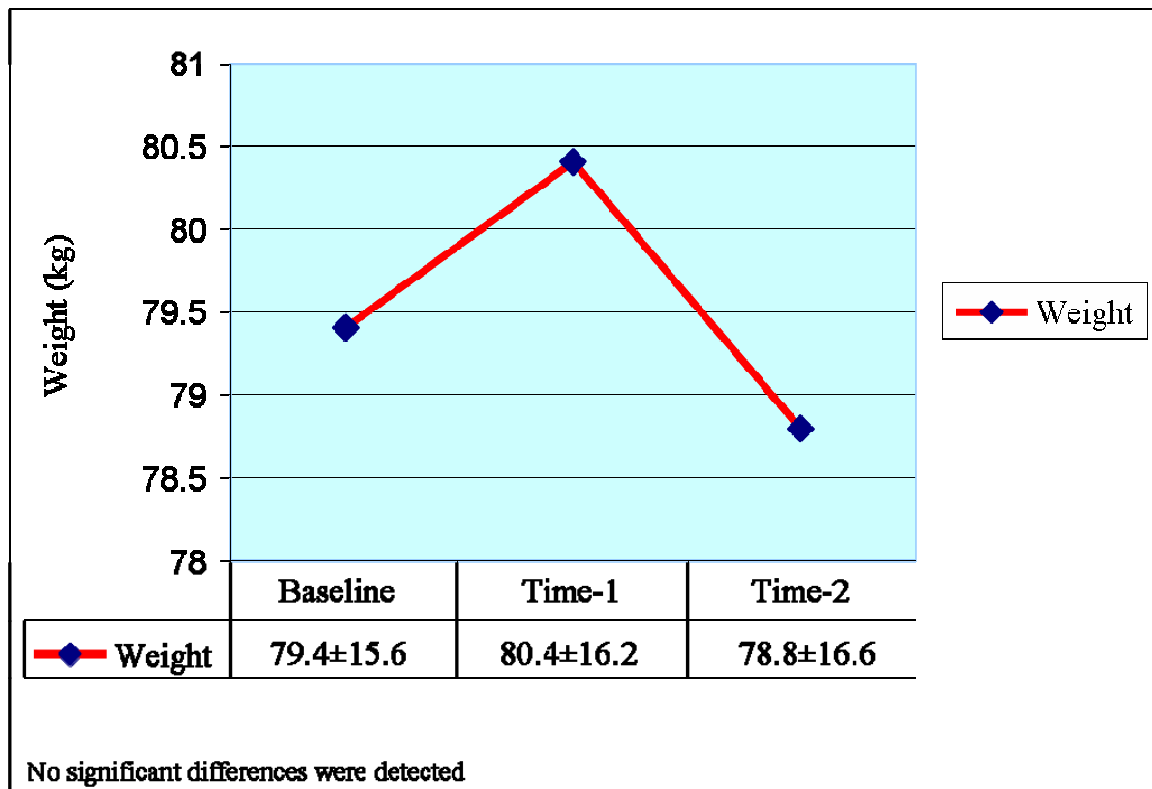
**Figure 11.** Resting energy expenditure and physical activity in rheumatoid arthritis patients on three times of assessment.



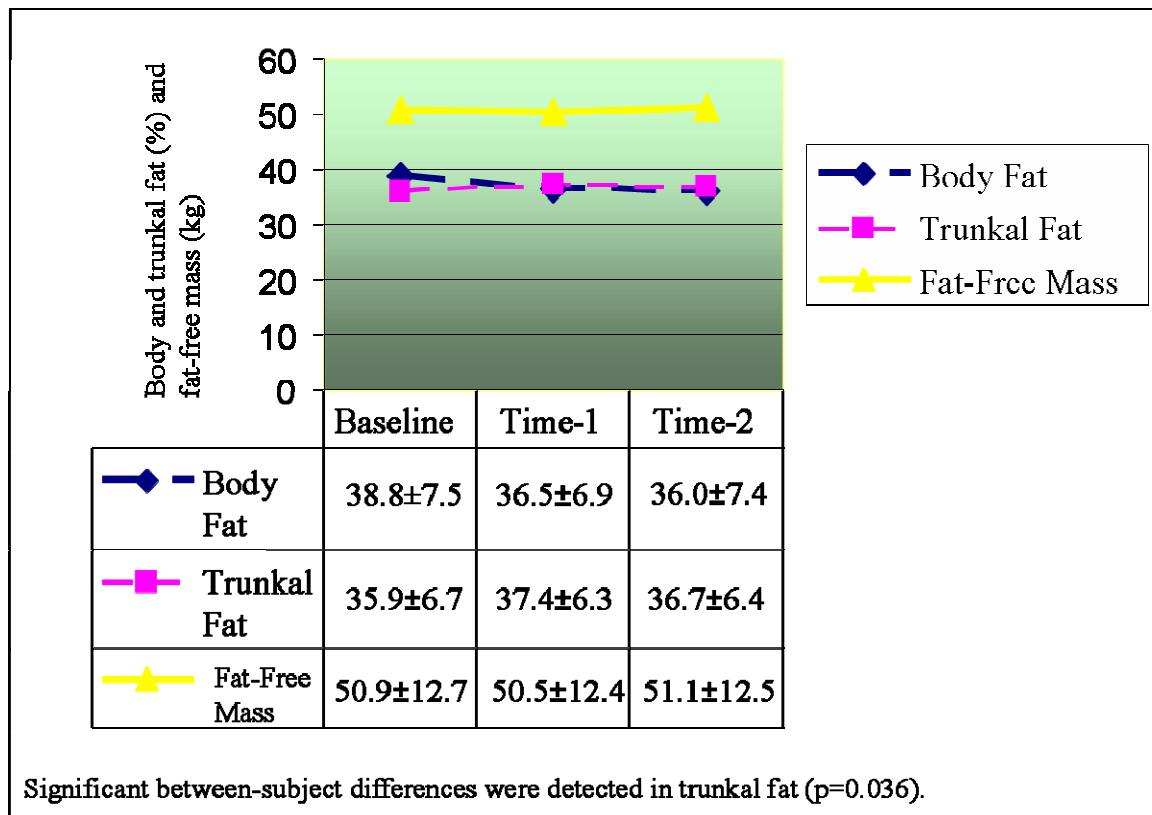
Mean $\pm$ std values and results from repeated measures for body composition assessment appear in Figures 12 and 13. Significant differences were observed only in

trunkal fat ( $p=0.036$ ) but not in any of the remaining body composition assessments ( $p>0.05$ ).

**Figure 12.** Weight in rheumatoid arthritis patients on the three times of assessment.

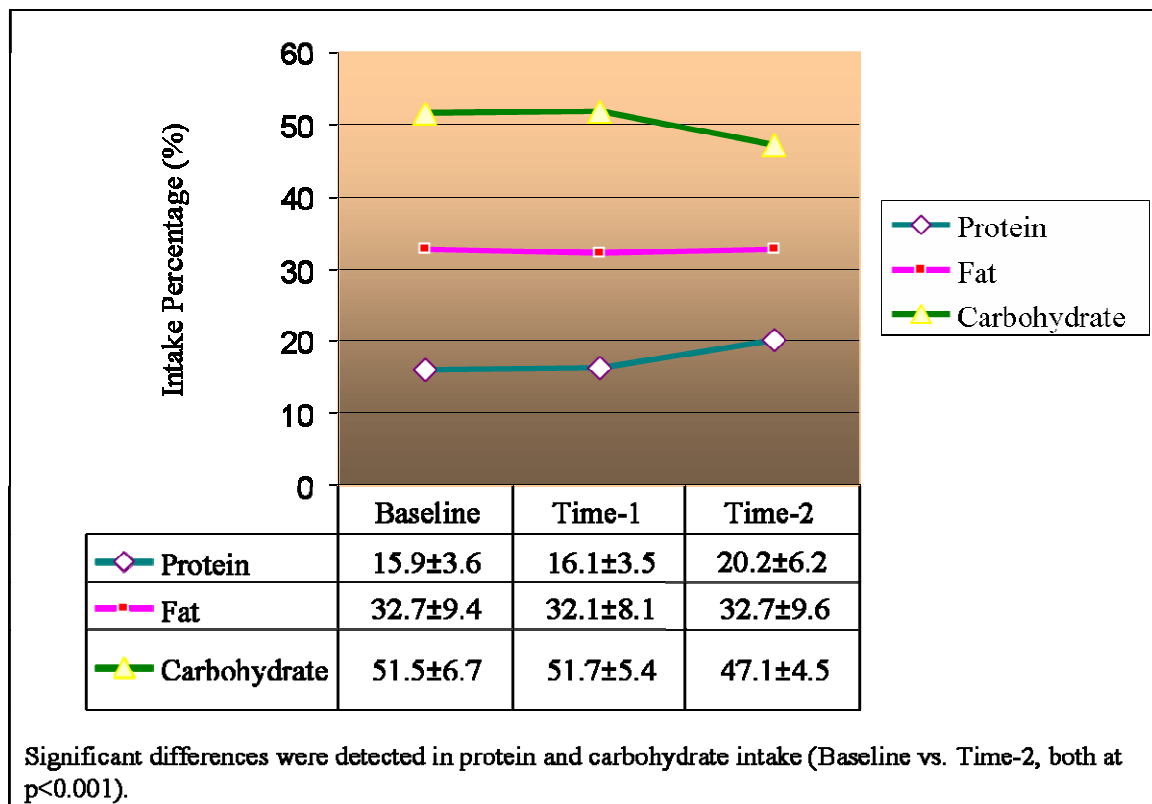


**Figure 13.** Body and trunkal fat and fat-free mass in rheumatoid arthritis patients on the three times of assessment.



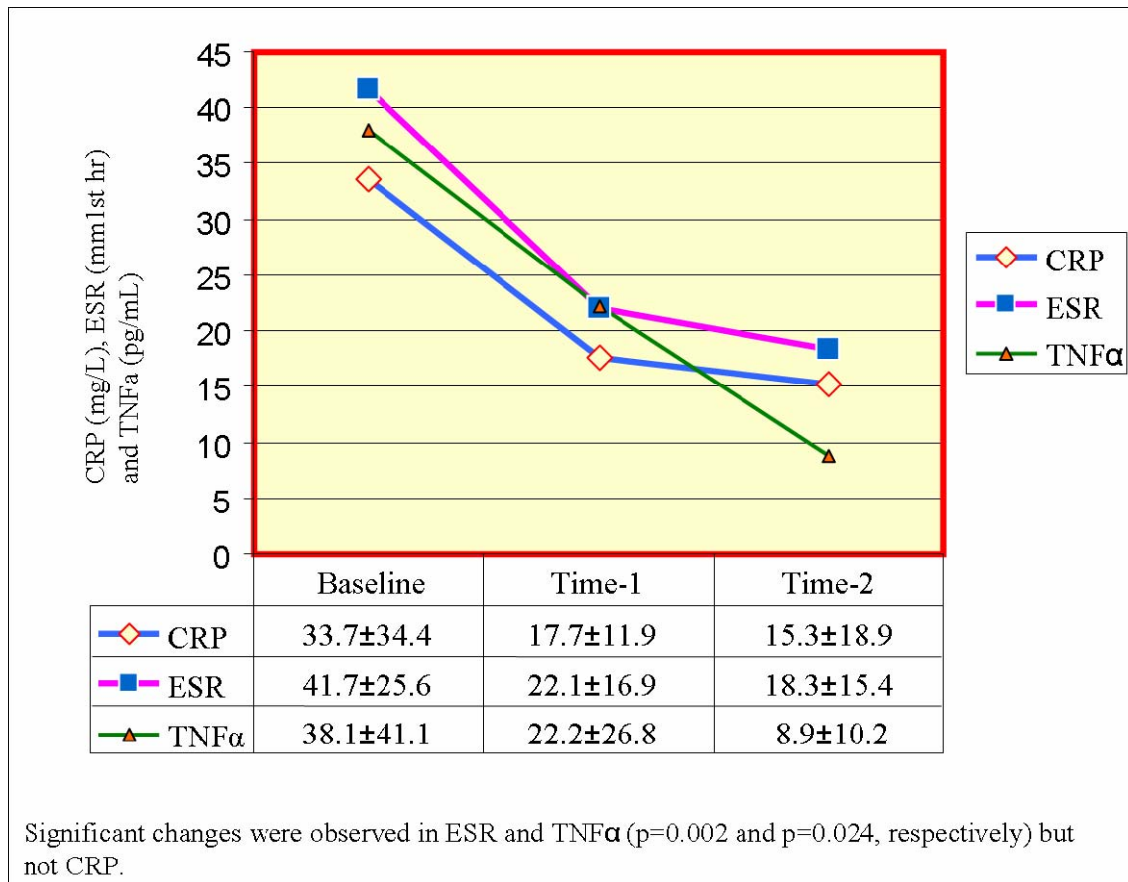
Protein intake was significantly increased within the studied 12-week course of treatment (Time 1:  $15.9 \pm 3.6$  vs. Time 2:  $16.5 \pm 3.5$  vs. Time 3:  $20.2 \pm 6.2$  %,  $p=0.001$ , Figure 14). Moreover, carbohydrate intake was significantly reduced (Time 1:  $51.3 \pm 6.7$  vs.  $51.7 \pm 5.4$  vs. Time 3:  $47.1 \pm 4.5$  %,  $p=0.001$ ) while fat did not change (Time 1:  $32.7 \pm 9.4$  vs.  $32.1 \pm 8.1$  vs.  $32.7 \pm 9.6$  %,  $p>0.05$ ). Increased protein intake was found to be a significant between-subject factor for the observed increase in REE ( $p=0.024$ ).

**Figure 14.** Percentage of protein, fat and carbohydrate intake in rheumatoid arthritis patients on the three times of assessment.



The exact values and comparisons between time-points for contemporary serological disease activity measurements (i.e., CRP and ESR) and levels of serum TNF $\alpha$  appear in Figure 15. Results revealed that ESR ( $p=0.002$ ) and TNF $\alpha$  ( $p=0.024$ ) significantly reduced while this was not the case for CRP ( $p>0.05$ ). Moreover, DAS28 (Time-1:  $5.66\pm0.7$  vs. Time-2:  $4.64\pm0.6$  vs. Time-3:  $3.59\pm0.7$ ,  $p=0.000$ ) and HAQ (Time-1:  $1.83\pm0.3$  vs. Time-2:  $1.54\pm0.6$  vs. Time-3:  $1.41\pm0.4$ ,  $p=0.000$ ) were also significantly reduced after 12-week on anti-TNF $\alpha$  medication.

**Figure 15.** C reactive protein, erythrocyte sedimentation rate and tumour necrosis factor alpha in rheumatoid arthritis patients on the three times of assessment.



#### 4. Discussion

The main aim of the present study was to investigate the effects of anti-TNFα therapy on rheumatoid cachexia. We have evaluated changes in two factors that characterise this metabolic abnormality: REE and body composition. We also evaluated physical activity and protein intake because they have a major impact on both REE and body composition (Kay and Fiatarone Singh 2006; Westerterp-Plantenga et al. 1999).

We chose to assess body composition via bioelectrical impedance because the method is non-invasive, quick and highly reproducible. It has previously been shown to have a high agreement with the total body water method in individuals with RA

(Heitmann et al. 1994). We also evaluated validity of bioelectrical impedance against skin-fold thickness in a pilot study in 22 local RA patients (72.7% female; age  $45.1 \pm 11.8$  yrs; weight  $68.0 \pm 15.9$  kg; height  $164.0 \pm 6.7$  cm): ANOVA revealed no significant differences ( $p=0.2$ ) and a high correlation ( $r=0.88$ ,  $p=0.000$ ) between these two methods.

Previous reports in the literature have revealed that REE is significantly increased in patients with rheumatoid arthritis because the disease is accompanied by excessive TNF $\alpha$  production which results in protein degradation and subsequent enhanced fat deposition, leading to functional disability and compromised immune function (Roubenoff et al. 1994; Roubenoff et al. 1992). It has been suggested that initiation of anti-TNF $\alpha$  therapy may reverse the process of rheumatoid cachexia (Metsios et al. 2006; Rall and Roubenoff 2004). Without investigating factors that may significantly altered body composition (i.e. REE, physical activity, and protein intake) a recent study (Marcora et al. 2006) found that fat (%) and FFM (kg) measures do not change in patients with early RA on anti-TNF $\alpha$  after 12 weeks. Our findings are similar to that study, even though our patients had longstanding RA. In addition, our data showed a significant variation in the percentage of trunkal fat within the three times of assessment. Bioelectrical impedance may be an accurate method in the estimation of total body fat and FFM, however, it does not ensure validity in the assessment of trunkal fat and hence, no firm conclusion for could be drawn for this change based on the current data.

Our study provides further important information since we have also assessed REE, physical activity, and protein intake. REE remains elevated, rather than reduced, as would be expected due to enhanced RA-related hypercatabolism, and this may be attributed to the significant increase in physical activity, a fact also supported by the literature (Gilliat-Wimberly et al. 2001; Toth and Poehlman 1996). It is possible that

maintenance of the elevated REE values detected in our participants, may have been predominantly due to the long term rather than the acute effect of exercise, since participants avoided physical activity for 72 hours before the REE assessment. In fact, REE can be increased even if FFM is maintained at the same levels (Poehlman and Danforth 1991) – which is also the case with our data – because FFM in individuals who engage in physical activities is metabolically more active compared to non-exercising counterparts (Withers et al. 1998). Another possible explanation for the observed REE elevation is the concomitant increase in protein intake, which was found to be a significant within-subject factor influencing REE. This is in line with relevant research findings suggesting that increased protein intake causes an elevation in REE due to the enhancement of muscle anabolic processes (Agus et al. 2000; Westerterp-Plantenga et al. 1999).

As expected disease activity significantly dropped as a response of anti-TNF $\alpha$  treatment whereas physical function significantly improved. We have used various variables to assess the changes in disease activity and all, except CRP, were markedly improved as a result of anti-TNF $\alpha$ . The assessment of CRP reflects the evaluation of the acute whereas ESR the long-term inflammatory load, since CRP kinetics are much faster compared to ESR. The fact that CRP did not change significantly was probably the result of the increased acute inflammatory load of three patients (on both 2 and 12 weeks) that coincided with the days of the assessment. These increased CRP values have probably shifted the CRP mean value upwards, causing the non-significant change in CRP throughout this 12-week period.

The fact that anti-TNF $\alpha$  therapy is associated with a significant increase in physical activity and a significant decrease in systemic inflammation in RA patients is

very encouraging. It is well-established that RA associates with increased cardiovascular mortality (del Rincon et al. 2001; Goodson et al. 2005; Kitas and Erb 2003) and excess recurrent cardiac events (Douglas et al. 2006) and this may relate both to classical cardiovascular risk factors (Erb et al. 2004) and to systemic inflammation (Kitas and Erb 2003; Maradit-Kremers et al. 2005; Stevens et al. 2005). Exercise has proved a very effective regimen for reducing mortality from cardiovascular disease (Adamu et al. 2006; Haennel and Lemire 2002) and anti-TNF therapy appears to associate with a reduction of cardiovascular mortality in RA, probably by also inhibiting systemic inflammation (Jacobsson et al. 2005).

The present study is limited by the lack of a control group on alternative disease-modifying therapies, and a somewhat unusual RA sample (with an equal female to male ratio). Within these limitations, we conclude that, in the short term, anti-TNF $\alpha$  treatment appears to prevent rheumatoid cachexia, reduce disease activity, improve physical function and increase physical activity. Despite that, abdominal fat deposition may not be arrested. The exact reasons and net metabolic and cardiovascular effects of these phenomena need to be assessed in detail in future studies.



## CHAPTER 5: GENERAL DISCUSSION

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### Chapter 1

In the first part of this work, we aimed to assess whether existing REE predictive equations were accurate in patients with RA: the results clearly showed that they are not. This was established, not only from the mean differences between measured and predicted REE values, but also on appropriate statistical analyses specifically for assessing agreement. In the control group, however, only a minimal number of the same equations resulted in bias between actual and predicted REE. This was not a surprising finding since the existing REE formulae have all been developed from data deriving from the normal population. Hence, they do not take into account the systemic inflammatory load, which may accompany the disease and disturb normal metabolic processes. Our data, in concert with previously published studies (Roubenoff et al. 1994; Roubenoff et al. 1992) support this; it was found that markers of contemporary disease activity significantly correlated with REE. We have, therefore, developed one prediction equation, which is specific for calculating REE in patients with RA.

Disease activity is assessed in RA patients quite frequently, using laboratory estimations of either ESR or CRP. CRP has much quicker kinetics of change (~48 hours) than ESR (~2 weeks), so it is thought to be a better marker of current / acute inflammation. Moreover, bioelectrical impedance is an apparatus commonly used in the clinical practice. The development of the equation incorporating FFM and CRP, can provide accurate estimation of REE in RA as well as easy and continuous monitoring of REE with the help of the current equation. REE can be monitored every time that the rheumatologist/nutritionist/health professional has knowledge of FFM and CRP values.

This, in turn could improve the disease outcomes leading to more effective patient management. Knowledge of resting metabolism in RA is important; increased REE indicates that metabolism is disturbed, predominantly by disease processes leading to catabolism. Hence, such periods of enhanced disease activity, which are frequent in RA, may require closer attention with regards to nutritional support. Increased protein intake should be advised to counterbalance the protein catabolism and restore the equilibrium between energy intake and expenditure in combination with nutrients that do not aggravate the disease symptoms. Moreover, patients experiencing more frequent disease exacerbations have less FFM compared to those with a more stable disease course. Given that loss of FFM compromises strength, balance, quality of life and increases mortality rates, attention has to be paid in educating RA patients regarding interventions that may inhibit muscle wasting, such as exercise and nutritional support.

## Chapter 2

The second chapter was an observational study on the effects of smoking on REE in RA patients; we have also assessed disease related outcomes such as health and number of swollen/tender joints. Results revealed that REE is increased in smokers compared to non-smokers and also accompanied by worse disease outcomes, assessed by both HAQ and DAS28.

A consistent finding of the literature is that smoking increases REE in the normal population, so this was expected to happen also in RA patients, even if the difference was not of sufficient magnitude to be detectable under the influence of other important confounders, such as systemic inflammation. The significance of our findings lies on this fact: RA patients experience hypermetabolism and become cachectic due to disease-related biological processes, a phenomenon also evident in the present

participants. This worsens disease outcomes, deteriorates quality of life and leads to increased mortality. Our data show that smoking further increases REE and may thus enhance these phenomena. This provides further evidence that smoking cessation should be one of the mainstay of management of patients with RA, and this may be facilitated by specific psycho-educational interventions available in the community or the healthcare environment. In addition, our findings raise an important issue: if REE is further enhanced by smoking leading to loss of FFM, it is necessary that smoking RA patients should be advised to commence specific nutritional support regimes, since, for example, overfeeding can stop FFM wasting and restore normal protein synthesis in various diseases. Few rheumatology units in the West Midlands, UK (i.e. Russell's Hall Hospital and Corbett Hospital, both in Dudley Group of Hospitals, NHS Trust), have, to our knowledge, active smoking cessation programmes and this may need to be included as part of the standards of care as outlined by the Arthritis and Musculoskeletal Alliance. Our findings will be incorporated into the adverse effects of smoking and reinforce its adverse effect on the health of RA patients while particular emphasis will be given to the nutritional support.

### Chapter 3

Our third study was aimed to investigate if anti TNF $\alpha$  treatment could reverse the RA-related hypermetabolism, since at least part of this has been attributed to the excessive production of TNF $\alpha$ . The findings of the third Chapter suggest that REE, physical activity, protein intake and disease-related outcomes (DAS28, HAQ, CRP) change significantly after three months of anti-TNF $\alpha$  while this was not the case for the studied body composition assessments (e.g., BMI, fat, FFM). The fact that REE remains elevated after 12-weeks of anti-TNF $\alpha$  treatment suggests that hypermetabolism is maintained;

however, we have investigated the factors that may cause such a change. Our results reveal that despite the maintenance of FFM (which may significantly alter REE), physical activity and increased protein intake accounted for the increased REE values.

A more thorough analysis of the dietary data would further strengthen the current findings, since specific nutrients may significantly inhibit enhanced catabolism; however, we have faced problems with the software that was available from the University of Wolverhampton, since the relevant company stopped the production of the software. Hence, we used a simpler software for nutritional analysis which could only estimate percentages of the major components of food intake. In addition, as discussed in the relevant chapter, our results are limited by the lack of a control group, which would allow for a more clear understanding of the results. However, the same-subject analysis adopted is a valid statistical method for the current study design and interpretation of the results. We decided to adopt this methodological design because of the difficulty to recruit patients on anti-TNF $\alpha$ ; indeed, this trial lasted 1.5 years. Initiation of the relevant treatment indicates that all previous medications were not tolerated hence these patients had more days where the disease was causing swelling and pain compared to other RA patients (in whom the medication controlled symptoms).

It is worth noting that the changes in protein energy intake were observed in pragmatic settings, since we did not motivate/inform our patients to change their physical activity and diet throughout the 12-week course of the experiment. Therefore, the increased REE values may have resulted as a response to increased exercise and protein intake, a phenomenon well described in the literature. It seems, therefore reasonable to suggest, that optimization of education towards more active lifestyles as well as diet

interventions in anti-TNF $\alpha$  patients will contribute to improved metabolic disease-related outcomes.

## CHAPTER 6: LIMITATIONS

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The limitations of the present study are:

- ❖ All biochemical assessment for the present projects were performed in the Pathology Laboratory, Dudley Group of Hospitals, NHS trust. As such, we did not gathered data regarding intra and inter-individual co-efficients for all biochemical parameters measured
- ❖ The lack of a power calculation before initiation of the first study. This would allow for a sufficient number of RA patients and thus making our new REE equation more valid
- ❖ Lack of accurate matching of RA patients and controls in the first study
- ❖ Validation of the new equation in a different sample of RA patients
- ❖ The use of an equal non-RA control group consisting of both smokers and non-smokers in the second study
- ❖ The small number of controls in second study
- ❖ The inclusion of ex-smokers in the second study, since the number of ex-smokers that was present in our RA cohort was very small
- ❖ The use of a more valid apparatus for the assessment of body composition (i.e. dual-energy x-ray absorptiometry)
- ❖ The lack of a high-sensitivity CRP analysis.
- ❖ The lack of a valid food diary as well as an appropriate dietary software for the analysis of the collected food diaries
- ❖ The rather short follow-up period (i.e. 12-weeks) in the third study

- ❖ The lack of a control RA group on alternative non-biological medicine in the third study
- ❖ Using a power calculator (DSS Research Power Calculator) we found that for CRP, the sample size in the third study ( $n=20$ ) gives us a power of 68%. To achieve a power of 80%, the sample size of participants should have been  $n=29$ .

## CHAPTER 7: SUGGESTIONS FOR FUTURE RESEARCH

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A future topic for further research, which arises from the first study, is the validation of the newly developed equations in a large sample of RA patients. The current sample was insufficient to both develop and validate the existing equations. In addition, validation is always subject to improvement via the inclusion of larger sample sizes. Another suggestion for future research is the development of such equations in other diseases with a significant, systemic, chronic inflammatory component, such as inflammatory bowel disease.

From the second Chapter an interesting future research project would be to investigate if RA smokers and non-smokers have different body composition proportions, i.e., FFM and fat mass and also how much this affects disease outcomes and functional capacity. Based on the present findings, RA smokers will have less FFM compared to non-smokers, a phenomenon probably caused and/or attributed to the combination of RA-related hypermetabolism in combination with smoking. In addition, it would be worth investigating if smoking cessation restores normal metabolism in RA. As mentioned above, some rheumatology units now include smoking cessation programmes where RA patients have quit smoking and are monitored regularly about their smoking habits; hence, an excellent area for research is the effects of smoking cessation on body composition and REE as well as the biological mechanisms (e.g., changes in thyroid hormones and catecholamines) that may contribute to this. Moreover, no studies to date have investigated the effects of smoking cessation on disease activity measures; based on the findings of this study, it would significantly improve disease outcomes.



The finding of our third study suggests that biological treatment with anti-TNF $\alpha$  may not significantly suppress REE. This generates the question regarding the effects of the other available biologic treatment on metabolism of RA patients. More specifically, interleukin-6 receptor antagonist and interleukin-1 receptor antagonist treatments block pro-inflammatory cytokines that act synergistically and cause rheumatoid cachexia. Hence, it remains to be investigated if they can control cachexia in the RA population by suppressing the levels of the relevant pro-inflammatory cytokines. Moreover, diet and exercise regimens alone or in combination with biological treatment, are potential interventions that may control this metabolic abnormality not only in RA, but in other populations with chronic inflammatory arthritis. From this work, we also found that protein intake and physical activity directly affect REE: hence, an interesting topic for future investigation is the potential effects of exercise in combination with appropriate diet on REE and body composition in RA patients immediately after diagnosis of RA. No studies have so far investigated this topic, since the traditional recommendation of rheumatologists was exercise restriction – owing to concerns about enhancing the joint inflammation and joint damage – while no further advice is currently provided regarding the beneficial effects of nutrition. If patients are appropriately educated and regularly advised/motivated to adhere to an active lifestyle and appropriate diet, they would not undergo this rapid muscle wasting.

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## APPENDIX 1



The Dudley Group of Hospitals



NHS Trust

### INFORMATION SHEET

Title of Research: Investigation of the accuracy of predictive equations to estimate Resting Energy Expenditure (REE) in patients with Rheumatoid Arthritis (RA).

Principal Investigator: Antonis Stavropoulos-Kalinoglou, MSc  
University of Wolverhampton  
(0121) 456 1302

Co-Investigators: George Metsios, MSc  
University of Wolverhampton  
(0121) 456 1302

Dr George Kitas, PhD, FRCP  
Rheumatology Research Unit  
Corbett Hospital  
(01384) 244 842

**Hospital and university regulations require us to obtain signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing the Consent Form.**

#### **Introduction to the research and invitation to participate in our study**

You are invited to participate in our study which aims to establish a better way for assessing resting energy expenditure (i.e., the energy required to maintain the functions of the body at rest) in patients with rheumatoid arthritis. You do not have to take part in the study. Your future treatment or care will not be affected if you decide to do so or if you decide to withdraw at any time from the study.

#### **What is the research study about?**

There are several calculations for predicting resting energy expenditure. These are based on healthy populations who are different from people with rheumatoid arthritis. The aim of this study is to find calculations that would be correct for patients with rheumatoid arthritis.

**What will I have to do?**

We need to measure your body fat and your resting energy expenditure. This will be done only once and will last no more than 2 hours.

**What will I have to do before the measurements?**

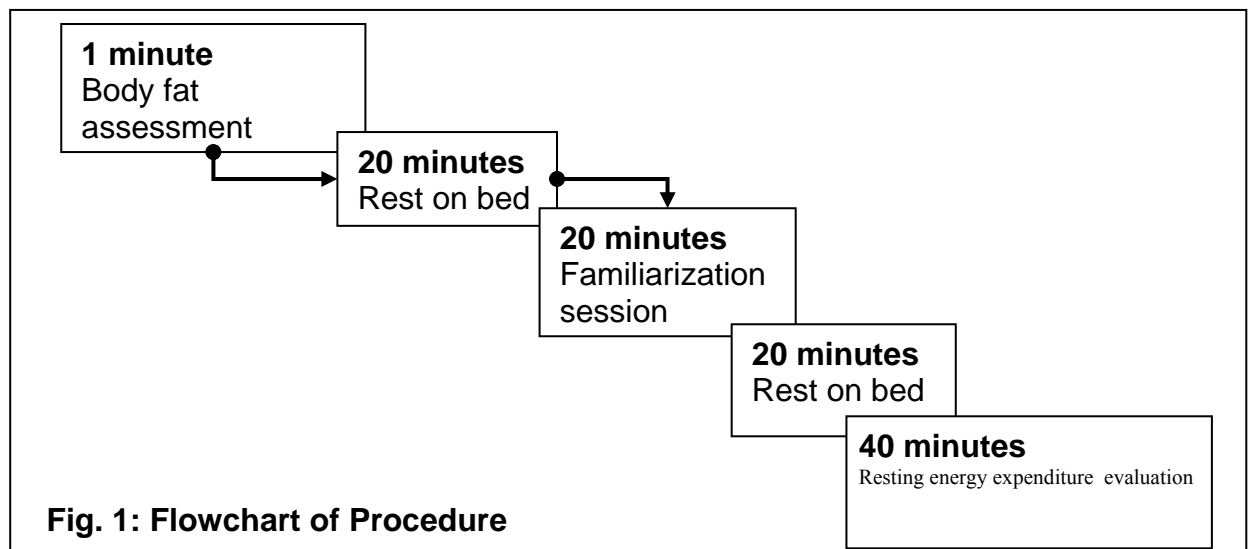
Fast overnight for 12 hours (as you would do if you had your blood sugar or cholesterol checked); avoid a lot of physical activity (e.g. running, playing sports) for 72 hours prior to the test; not to change your usual diet and medication; on the day of the test, come to the hospital first thing in the morning (i.e., about 8.00 o'clock).

**What does the test involve?**

The testing procedure is described in figure 1.

- 1) Body Composition:** Upon your arrival at the clinic, you will need to stand on a set of scales (similar to those used to measure your weight) for just one minute.
- 2) Resting Energy Expenditure:** The way we measure your resting energy expenditure is by monitoring your oxygen uptake over a period of time. To do this we will require you to wear a hood or a mask. There are 4 stages to take your resting energy expenditure measurement. This will take 1 hour and 40 minutes. During this time you will be resting but not asleep.
  - a. You will lie on a bed for 20 minutes.
  - b. We will then place either a hood or a mask over your head (see photo) for another 20 minutes so that you can get used to wearing the equipment. You will be able to breathe as normal whilst wearing them.
  - c. You will then rest on the bed for another 20 minutes without wearing the hood or the mask.
  - d. We will then replace the hood or the mask and measure you breathing for a period of 40 minutes.
- 3) Disease Activity:** Disease activity (how many painful and swollen joints you have) will be assessed in the usual way (i.e. by gently squeezing your joints to feel whether they are swollen so you can tell us whether they are painful).





### **What are the benefits from this study?**

It is unlikely that you will have a direct, personal benefit from taking part in this study. However, the information we will gather from this, will help us understand better the needs of patients with rheumatoid arthritis. This is important, because it has consequences for their general well-being and survival. With this knowledge, we hope to be able to help people with rheumatoid arthritis in the future.

### **What are the risks?**

You may feel uncomfortable when using the transparent hood or mask that is necessary for measurement of your resting energy expenditure. If this is the case, and you are unable to tolerate the test, we will stop it at that point. There are no other risks associated with this study.

### **What happens if I do not want to take part or change my mind during the study?**

If you decide to participate, this is entirely of your own free will. If at any point and for any reason you do not want to carry on then you may stop. Your treatment does not depend on your participation in this study.

### **What happens to the information?**

All information obtained from you will be kept strictly confidential. This information will be identified according to a code number known only to those directly involved with this project. We will write to your GP to let him/her know that you have participated.

### **Who else is taking part?**

All patients with rheumatoid arthritis in the Dudley Rheumatology Department will be asked to participate, irrespective of disease activity at the time of the measurement. To produce good results, we need more than 50 patients.

### **What if something goes wrong?**

If something goes wrong during testing, we will stop the testing and a doctor will examine you and act accordingly. If anything goes wrong with your treatment, this will be dealt with in the usual way, using the resources of the department of rheumatology and your general practitioner.

**What happens at the end of the study?**

We will analyse the results and come to some conclusions. We hope to be able to present these in national and international meetings and to publish the results in scientific journals. Any such publication will not identify individuals, but will be for the whole group of people that took part in the study. If you wish, we will send you a summary of our findings, and these will also be displayed in public areas of the Rheumatology Department in the hospital.

**If I take part, do I get paid?**

No, but if you wish, we will reimburse your travel expenses.

**What if I have more questions or do not understand something?**

If you have any further questions, do not hesitate to ask the research assistant conducting the study (i.e., Antonis), his colleague (George), the research nurse (Sister Debbie Mitton) or Dr. George Kitas. You can also call our research or routine telephone line on 01384-244759 or 01384-244789; leave a message and we will call you back.

**What happens now if I decide to take part?**

If you want to take part please contact Antonis (0121- 456 1302) or the Rheumatology Clinic (01384-244 789) to make an appointment.

**This Information Sheet is yours to take home**

Title of Research: Investigation of the accuracy of predictive equations to estimate Resting Energy Expenditure (REE) in patients with Rheumatoid Arthritis (RA).

Principal Investigator: Antonis Stavropoulos-Kalinoglou  
University of Wolverhampton  
(0121) 456 1302

Co-Investigators: George Metsios, MSc  
University of Wolverhampton  
(0121) 456 1302

Dr George Kitas, PhD, FRCP  
Rheumatology Research Unit  
Corbett Hospital  
(01384) 244 842

**I certify that I have read and understand the statement of procedure described in the Information Sheet and agree to participate as a subject in the research described therein. I understand that I may discontinue participation at any time without penalty or loss of any benefit to which I might otherwise be entitled. I certify that I am at least eighteen years of age.**

_____	_____	____/____/____
Print Your Name Here	Sign Here	Date
_____	_____	____/____/____
Mane of Witness	Sign Here	Date

**A copy of this Consent Form will be made for you to take home.**

## **Recruitment Advertisement**

Title of Research: Investigation of the accuracy of predictive equations to estimate Resting Energy Expenditure (REE) in patients with Rheumatoid Arthritis (RA).

Principal Investigator: Antonis Stavropoulos-Kalinoglou  
University of Wolverhampton  
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Co-Investigators: George Metsios, MSc  
University of Wolverhampton  
(0121) 456 1302

Dr George Kitas, PhD, FRCP  
Rheumatology Research Unit  
Corbett Hospital  
(01384) 244 842

## **Our Study**

You are invited to participate in our study which aims to establish a better way for assessing resting energy expenditure (i.e., the energy required to maintain functions of the body at rest) in patients with rheumatoid arthritis. You are under no obligation to take part in the study. Furthermore, your future treatment or care will not be affected if you decide to do so or if you decide to withdraw at any time from the study.

## **What Do I Need To Do?**

If you want to take part please contact the research assistant, Antonis (0121-456 1302), or the rheumatology research nurse, Debbie Mitton (01384-244789). We will make an appointment at a convenient for you time in order to provide you with more information about the study.

## APPENDIX 2



The Dudley Group of Hospitals



### INFORMATION SHEET

Title of Research: Acute and long-term effects of effective control of systemic inflammation (using anti-TNF therapy) on body composition and resting energy expenditure in patients with rheumatoid arthritis.

Principal Investigator: George Metsios, MSc  
University of Wolverhampton  
(0121) 456 1302

Co-Investigators: Antonis Stauroopoulos-Kalinoglou, MSc  
University of Wolverhampton  
(0121) 456 1302

Dr George Kitas, PhD, FRCP  
Rheumatology Research Unit  
Corbett Hospital  
(01384) 244 842

**Hospital and university regulations require us to obtain signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing the Consent Form.**

#### **Introduction to the research and invitation to participate in our study**

You are invited to participate in our study which aims to investigate the effects of anti-TNF therapy on body fat and resting energy expenditure (i.e., the energy required to maintain bodily functions at rest) in patients with rheumatoid arthritis. You are under no obligation to take part. Your future treatment or care will not be affected if you decide not to take part or if you decide to withdraw at any time from the study.



**What is the research study about?**

The purpose of this study is to investigate the acute (after two weeks) and long-term (after three and six months) effects of anti-TNF therapy on body fat and resting energy expenditure in patients with rheumatoid arthritis. Our goal is to learn more about strategies that have beneficial effects on both the increased metabolism and body fat in individuals with rheumatoid arthritis.

**What will I have to do?**

This research will monitor the way your body responds to the anti-TNF therapy. For this reason we will measure your body fat, your metabolism while you rest, and blood for testing (which is anyway part of your routine), during four separate visits to the clinic. The same procedures will be conducted 4 times in a period of 6 months, with each session lasting no more than 2 hours.

The first session will be conducted on the day that you receive your first anti-TNF medicine, while the second and the third will be after a two-week and a three-month period, respectively. The last visit will be after 6 months. All four sessions will be conducted during the morning and they will be exactly the same involving the following assessments:

**4) Body Composition:** Upon your arrival at the clinic, you will need to stand on a set of scales (similar to those used to measure your weight) for just one minute.

**5) Resting Energy Expenditure:** The way we measure your resting energy expenditure is by monitoring your oxygen uptake over a period of time.

To do this we will require you to wear a hood or a mask. On your first visit you will follow a larger routine so that you become familiar with the equipment. There are 4 stages to take your resting energy expenditure measurement. This will take 1 hour and 40 minutes. During this time you will be resting but not asleep.

- a. You will lie on a bed for 20 minutes.
- b. We will then place either a hood or a mask over your head (see photo) for another 20 minutes so that you can get used to wearing the equipment. You will be able to breathe as normal whilst wearing them.
- c. You will then rest on the bed for another 20 minutes without wearing the hood or the mask.
- d. We will then replace the hood or the mask and measure you breathing for a period of 40 minutes.

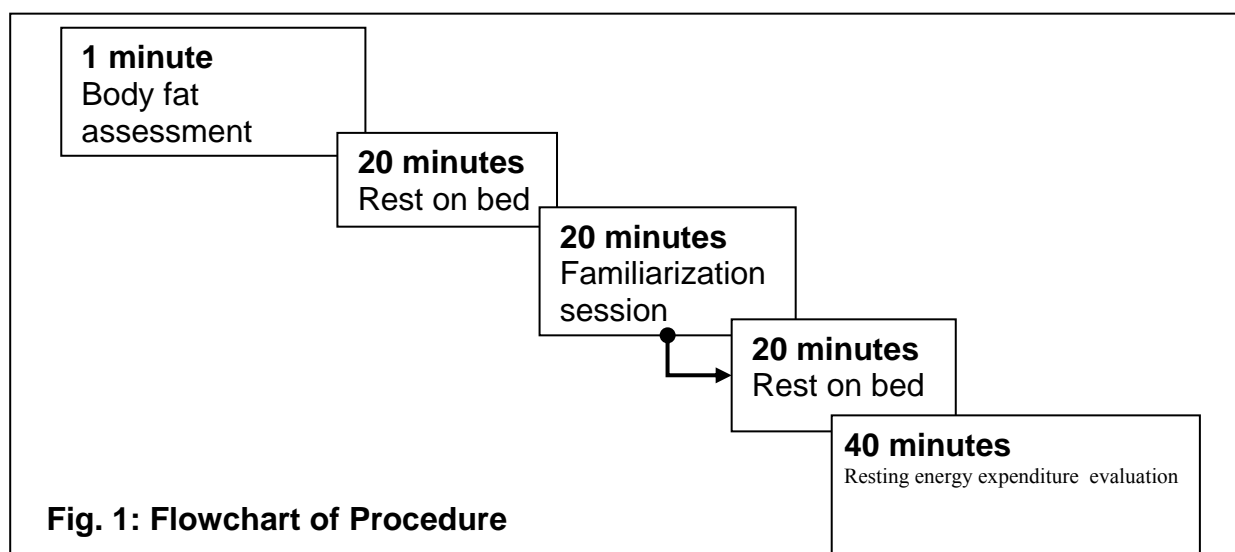


On your second and third visits the session will last just 1 hour. You will rest for 20 minutes and we then measure your breathing for 40 minutes.

**6) Blood Sampling:** As part of your routine due to your anti-TNF therapy you will have to give a blood sample. From this sample we will perform additional analyses to measure levels of specific chemicals in your blood.

**7) Physical Activity Questionnaire:** We are interested in finding out the kinds of physical activities that you do as part of your everyday life. The questions will ask you about the time you spent being physically active in the last 7 days. You will fill in the questionnaire the times that you are at the clinic for your routine check.

**8) Food Diary:** When you attend the clinic we will ask you to hand in a diary (provided by us) describing in detail what you ate for three consecutive days during the week.



**What will I have to do before the measurements?**

- An overnight fasting for 12 hours.
- Avoid strenuous physical activity 72 hours prior to visits.
- Come to the hospital first thing in the morning.
- Not change anything in your diet.

**NOT change you medication.**

**What are the benefits ?**

By your taking part in this study we will be able to monitor exactly how your body will respond to the anti-TNF therapy. In addition, the information we get from this study may help us to better treat future patients with rheumatoid arthritis and provide better understanding about the nutritional needs of rheumatoid arthritis patients before, during and after such a therapy.

**What are the risks ?**

You may feel slight discomfort when the needle is inserted into a vein in your arm so that we can collect blood. Also, you may feel slight discomfort when the transparent hood or the mask is placed into position.

**What happens if I do not want to take part or change my mind during the study?**

If you decide to participate, this is entirely of your own free will. If at any point and for any reason you do not want to carry on then you may stop. Your treatment does not depend on your participation.

**What happens to the information ?**

All information obtained from you will be kept strictly confidential. This information will be identified according to a code number known only to those directly involved with this research project. We will write to your GP to let him/her know that you have participated.

**Who else is taking part ?**

This study refers to patients with RA that are about to embark on an anti-TNF $\alpha$  therapy course. Both men and women, of all ages, from the Dudley Rheumatology clinics will be asked to participate, irrespective of disease activity at the time of the measurement.

Approximately 16 patients will be invited to take part. The entire testing procedure will last six months; during that period four evaluation sessions will be completed. Each one of those four sessions will last no more than 2 hours.

**What if something goes wrong ?**

If something goes wrong we will stop the testing and a doctor will examine you.

**What happens at the end of the study ?**

We will analyse the results and come to some conclusions. We hope to be able to present these in national and international meetings and to publish the results in scientific journals. Any such publication will not identify individuals, but will be for the whole group of people that took part in the study. If you wish, we will send you a summary of our findings, and these will also be displayed in public areas of the Rheumatology Department in the hospital.

**If I take part, do I get paid ?**

No, but if you wish, we will reimburse your travel expenses.

**What if I have more questions or do not understand something ?**

If you have any questions, do not hesitate to ask the research assistant conducting the study. If you have any questions either before or after participating in this study, you may also contact the director of the study, Dr George Kitas (01384-244842).

**What happens now if I decide to take part ?**

If you want to take part please contact George (Tel: 0121- 456 1302) his colleague (Andonis), the research nurse (Sister Debbie Mitton; Tel: 01384 244796) or Dr. George Kitas. You can also call our research or routine telephone line on 01384-244759 or 01384-244789; leave a message and we will call you back.

**This Information Sheet is yours to take home**

Title of Research: Acute and long-term effects of effective control of systemic inflammation (using anti-TNF therapy) on body composition and resting energy expenditure in patients with rheumatoid arthritis.

Principal Investigator: George Metsios, MSc  
University of Wolverhampton  
(0121) 456 1302

Co-Investigators: Antonis Stauropoulos-Kalinoglou  
University of Wolverhampton  
(0121) 456 1302

Dr George Kitas, PhD, FRCP  
Rheumatology Research Unit  
Corbett Hospital  
(01384) 244 842

**I certify that I have read and understand the statement of procedure described in the Information Sheet and agree to participate as a subject in the research described therein. I understand that I may discontinue participation at any time without penalty or loss of any benefit to which I might otherwise be entitled. I certify that I am at least eighteen years of age.**

\_\_\_\_\_/\_\_\_\_\_/\_\_\_\_\_  
Print Your Name Here                      Sign Here                      Date

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Mane of Witness                      Sign Here                      Date

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## **Recruitment Advertisement**

<u>Title of Research:</u>	Acute and long-term effects of effective control of systemic inflammation (using anti-TNF therapy) on body composition and resting energy expenditure in patients with rheumatoid arthritis.
<u>Principal Investigator:</u>	George Metsios, MSc University of Wolverhampton (0121) 456 1302
<u>Co-Investigators:</u>	Antonis Stauropoulos-Kalinoglou University of Wolverhampton (0121) 456 1302  Dr George Kitas, PhD, FRCP Rheumatology Research Unit Corbett Hospital (01384) 244 842

## **Our Study**

You are invited to participate in our study which aims to investigate the effects of anti-TNF therapy on body fat and resting energy expenditure (i.e., the energy required to maintain functions of the body at rest) in patients with rheumatoid arthritis. You are under no obligation to take part.

## **What Do I Need To Do ?**

If you want to take part please contact either:

George (0121-456 1302), or the rheumatology clinic (01384-244789)  
to make an appointment for your first session.

### APPENDIX 3

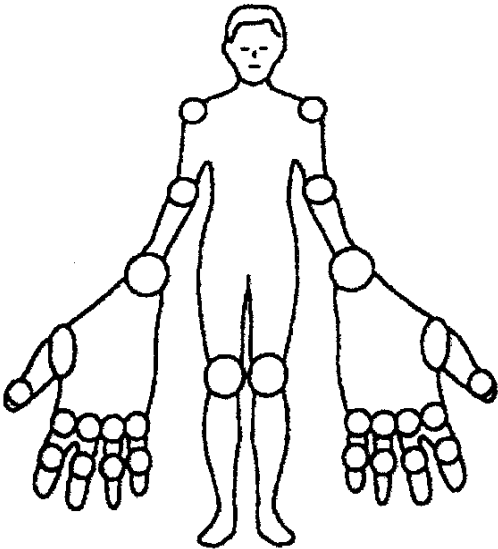
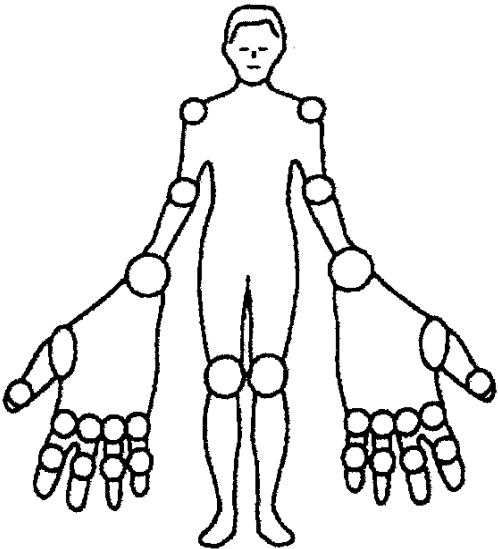
Please mark on the line above how your overall health has been in the last week

\_\_\_\_\_

**Worst ever health**

**Best ever health**

#### 28 Joint Count

Swollen Joints	Tender joints
	

Total.....

Total.....

## APPENDIX 4

### HEALTH ASSESSMENT QUESTIONNAIRE (HAQ)

**Date:**

**Patient Name:**

Please tick the one response which best describes your usual abilities over the past week

	Without ANY difficulty	With SOME difficulty	With MUCH difficulty	to do
<b>UNABLE</b>				
<b>1. DRESSING and GROOMING</b>				
Are you able to:				
a. Dress yourself, including tying shoelaces and doing buttons?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Shampoo your hair?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>2. RISING</b>				
Are you able to:				
a. Stand up from an armless straight chair?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Get in and out of bed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>3. EATING</b>				
Are you able to:				
a. Cut your meat?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Lift a full cup or glass to your mouth?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. Open a new carton of milk (or soap powder)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>4. WALKING</b>				
Are you able to:				
a. Walk outdoors on flat ground?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Climb up five steps?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**PLEASE TICK ANY AIDS OR DEVICES THAT YOU USUALLY USE FOR ANY OF THESE ACTIVITIES:**

Cane (W) <input style="width: 40px;" type="checkbox"/>	Walking frame(W) <input style="width: 40px;" type="checkbox"/>	Built-up or special utensils (E) <input style="width: 40px;" type="checkbox"/>
Crutches (W) <input style="width: 40px;" type="checkbox"/>	Wheelchair (W) <input style="width: 40px;" type="checkbox"/>	Special or built-up chair (A) <input style="width: 40px;" type="checkbox"/>

Devices used for dressing (button hooks, zipper pull, shoe horn)

☐

Other (specify).....

**PLEASE TICK ANY CATEGORIES FOR WHICH YOU USUALLY NEED HELP FROM ANOTHER PERSON:**

Dressing and Grooming

☐

Eating

☐

Rising

☐

Walking

☐

Please tick the one response which best describes your usual abilities over the past week

	Without ANY difficulty	With SOME difficulty	With MUCH difficulty	to do
--	---------------------------	-------------------------	-------------------------	-------

**5. HYGIENE**

Are you able to:

a. Wash and dry your entire body?

☐☐☐☐

b. Take a bath?

☐☐☐☐

c. Get on and off the toilet?

☐☐☐☐

**6. REACH**

Are you able to:

a. Reach and get down a 5 lb object  
(e.g. a bag of potatoes) from just  
above your head?

☐☐☐☐

b. Bend down to pick up clothing  
off the floor?

☐☐☐☐

**7. GRIP**

Are you able to:

a. Open car doors?

☐☐☐☐

b. Open jars which have been  
previously opened?

☐☐☐☐

c. Turn taps on and off?

☐☐☐☐

**8. ACTIVITIES**

Are you able to:

a. Run errands and shop?

☐☐☐☐

b. Get in and out of a car?

☐☐☐☐

c. Do chores such as vacuuming,

☐☐☐☐



housework or light gardening?

**PLEASE TICK ANY AIDS OR DEVICES THAT YOU USUALLY USE FOR ANY OF THESE ACTIVITIES:**

Raised toilet seat (H)	<input type="checkbox"/>	Bath seat (H)	<input type="checkbox"/>	Bath rail (H)	<input type="checkbox"/>
Long handled appliances for reach (R)	<input type="checkbox"/>				
Jar opener (for jars previously opened) (G)	<input type="checkbox"/>				

Other (specify) \_\_\_\_\_

**PLEASE TICK ANY CATEGORIES FOR WHICH YOU USUALLY NEED HELP FROM ANOTHER PERSON:**

Hygiene	<input type="checkbox"/>	Gripping and opening things	<input type="checkbox"/>
Reach	<input type="checkbox"/>	Errands and housework	<input type="checkbox"/>

## APPENDIX 5

### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

#### **Background on IPAQ**

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

#### **Using IPAQ**

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

#### **Translation from English and Cultural Adaptation**

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at [www.ipaq.ki.se](http://www.ipaq.ki.se). If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

#### **Further Developments of IPAQ**

International collaboration on IPAQ is on-going and an *International Physical Activity Prevalence Study* is in progress. For further information see the IPAQ website.

#### **More Information**

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at [www.ipaq.ki.se](http://www.ipaq.ki.se) and Booth, M.L. (2000).

*Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

## **PART 1: JOB-RELATED PHYSICAL ACTIVITY**

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

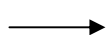
1. Do you currently have a job or do any unpaid work outside your home?

☐

Yes

☐

No



**Skip to PART 2: TRANSPORTATION**

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include travelling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like

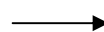
heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**?

Think about only those physical activities that you did for at least 10 minutes at a time.

\_\_\_\_\_ **days per week**

☐

No vigorous job-related physical activity



**Skip to question 4**

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

\_\_\_\_\_ **hours per day**

\_\_\_\_\_ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

\_\_\_\_\_ **days per week**

☐

No moderate job-related physical activity



**Skip to question 6**

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

\_\_\_\_\_ **hours per day**

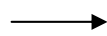
\_\_\_\_\_ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

\_\_\_\_\_ **days per week**

☐

No job-related walking



**Skip to PART 2:**

### **TRANSPORTATION**

7. How much time did you usually spend on one of those days **walking** as part of your work?

\_\_\_\_\_ **hours per day**

\_\_\_\_\_ **minutes per day**

### **PART 2: TRANSPORTATION PHYSICAL ACTIVITY**

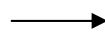
These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

\_\_\_\_\_ **days per week**

☐

No travelling in a motor vehicle



**Skip to question 10**

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

\_\_\_\_\_ **hours per day**

\_\_\_\_\_ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

\_\_\_\_\_ **days per week**

☐

No bicycling from place to place



**Skip to question 12**

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

\_\_\_\_\_ **days per week**

☐

No walking from place to place —————>

**Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY**

13. How much time did you usually spend on one of those days **walking** from place to place?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

***PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

\_\_\_\_\_ **days per week**

☐

No vigorous activity in garden or yard —————>

**Skip to question 16**

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

\_\_\_\_\_ **days per week**

☐

No moderate activity in garden or yard —————>

**Skip to question 18**

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

\_\_\_\_\_ **days per week**

☐ No moderate activity inside home → **Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY**

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

**PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY**

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

\_\_\_\_\_ **days per week**

☐ No walking in leisure time → **Skip to question 22**

21. How much time did you usually spend on one of those days **walking** in your leisure time?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

\_\_\_\_\_ **days per week**

☐ No vigorous activity in leisure time → **Skip to question 24**

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

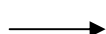
\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

\_\_\_\_\_ **days per week**

☐

No moderate activity in leisure time



**Skip to PART 5: TIME SPENT SITTING**

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

#### ***PART 5: TIME SPENT SITTING***

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

\_\_\_\_\_ **hours per day**  
\_\_\_\_\_ **minutes per day**

**This is the end of the questionnaire, thank you for participating.**